The Vascular Marker CD31 Also Highlights Histiocytes and Histiocyte-Like Cells Within Cutaneous Tumors

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ABSTRACT

Objectives: While useful in diagnosing angiosarcomas, CD31 can also highlight histiocytes within soft tissue tumors and lead to errors in diagnosis. We sought to determine how often CD31 highlights cutaneous histiocytomas and histiocytoma mimics.

Methods: We examined eight epithelioid cell histiocytomas (ECHs), 12 xanthogranulomas (XGs), nine cases of Langerhans cell histiocytosis (LCH), eight reticulohistiocytomas, 11 xanthomas, 29 atypical fibroxanthomas, nine granular cell tumors, four cases of angiolymphoid hyperplasia with eosinophilia, nine intradermal Spitz nevi, and nine angiosarcomas with antibodies directed against CD31, CD34, CD163, and factor VIII.

Results: CD31 marked cells in three of 12 XGs, four of nine cases of LCH, one of eight reticulohistiocytomas, one of 11 xanthomas, 10 of 29 atypical fibroxanthomas, four of four cases of angiolymphoid hyperplasia with eosinophilia, nine of nine angiosarcomas, zero of nine granular cell tumors, and zero of eight ECHs. CD34 and factor VIII were negative in all nonvascular cases.

Conclusions: Our results indicate that CD31 can mark lesional cells and imitate vascular tumors in cutaneous histiocytomas and histiocytoma mimics, an error that can be avoided by using a panel of antibodies.

CD31, also known as platelet endothelial cell adhesion molecule 1 (PECAM-1), is thought to be a sensitive and specific marker for vascular differentiation.1,2 It is a transmembrane glycoprotein expressed by endothelial cells and a variety of hematopoietic cells.3 It is often used alone or in conjunction with CD34 in the diagnosis of vascular tumors, such as angiosarcomas.4-7 It has, however, been shown that CD31 can pose a diagnostic pitfall when deep soft tissue tumors are evaluated, since it weakly stains intratumoral histiocytes.1 The frequency with which this problem occurs in a range of cutaneous neoplasms of histiocytic origin is not well studied. To investigate this further, we initiated a study of the expression of CD31 in a variety of cutaneous histiocytomas, histiocyte-rich tumors, tumors with histiocyte-like cells, and angiosarcomas and compared the results with those obtained using CD34 and factor VIII. CD163 was also tested concurrently to examine the correlation between CD31 and CD163 expression.
Materials and Methods

Case Selection

A total of 108 formalin-fixed, paraffin-embedded tissues were selected from the archives of the Department of Pathology at Stanford University based on the original diagnosis. The initial H&E-stained sections, as well as relevant immunohistochemical stains performed after biopsy, were reviewed to confirm the diagnosis in each case. The samples consisted of 12 xanthogranulomas (XGs), eight reticulohistiocytes, eight epithelioid cell histiocytomas, nine cases of Langerhans cell histiocytosis (LCH), 11 xanthomas, 29 atypical fibroxanthomas (AFXs), nine granular cell tumors, four cases of angiolymphoid hyperplasia with eosinophilia, nine intradermal Spitz nevi, and nine angiosarcomas. None of the cases of atypical fibroxanthoma were positive for S100 or cytokeratin markers (CKAE1 and/or CK5/6). This study was approved by Stanford University’s Institutional Review Board in accord with the ethical standards established by that institution.

Immunohistochemistry

Immunohistochemical studies were performed on 4-μm-thick conventional sections of formalin-fixed, paraffin-embedded tissue and were stained with antibodies directed against CD31, CD34, and factor VIII using a Ventana BenchMark XT system (Ventana Medical Systems, Tucson, AZ). Results for CD163 have been reported previously. The iView 3,3′-diaminobenzidine chromogen system (Ventana Medical Systems) was used for detection. Staining for CD31 localized predominantly to the membrane but occasionally stained the cytoplasm as well, especially in histiocytes. CD34 and factor VIII localized predominantly to the membrane, whereas CD163 primarily stained the cytoplasm. Normal vessels served as positive internal controls for CD31, CD34, and factor VIII, and these were present in all negative cases. Accompanying normal-spindled histiocytes served as positive internal controls for CD163, and skin epidermal cells served as negative internal controls for all antibodies. Immunoreactivity was scored as follows: strongly positive, intense staining of at least 20% of lesional cells; weakly positive, intense staining of at least 5% but less than 20% of lesional cells or faint or moderate staining of at least 20% of lesional cells; and negative, staining of less than 5% to no staining of lesional cells. Positive scoring consisted of the sum of cases scored as either strongly or weakly positive.

Results

Clinical information for patients in this study is listed in Table 2, and the results from the immunohistochemical studies with CD31, CD34, CD163, and factor VIII are listed in Table 3. The 12 cases of XG were characterized by dermal proliferations of histiocytes, foam cells, and varying numbers of Touton-type giant cells. The 29 cases of AFX were composed of a dermal mixture of pleomorphic spindle and epithelioid cells, histiocytes, and occasional multinucleated giant cells; at least one case was hemorrhagic and rich in vessels. The eight cases of reticulohistiocytoma contained histiocytes with large vesicular nuclei and giant cells with ground-glass cytoplasm. The eight cases of epithelioid cell histiocytoma showed numerous large epithelioid cells with cosinophilic cytoplasm and prominent vesicular nuclei. The nine cases of LCH were characterized by a dermal infiltrate of atypical histiocytic cells with large reniform nuclei. The 11 cases of xanthoma had dermal proliferations of small foam cells with pale cytoplasm and small vesicular nuclei. The nine cases of angiosarcoma were composed of both epithelioid and spindled cells but also were characterized by focal neoplastic vessels carving out dermal collagen with accompanying extravasated erythrocytes. In the nine cases of granular cell tumor, epithelioid cells with abundant granular cytoplasm infiltrated dermal collagen in an interstitial pattern. The four cases of angiolymphoid hyperplasia with eosinophilia (epithelioid hemangioma) had a proliferation of vascular channels lined by enlarged epithelioid cells admixed with lymphocytes and eosinophils. The nine cases of intradermal Spitz nevi contained groups of large spindled and epithelioid melanocytes that formed dermal nests and demonstrated maturation with increasing dermal descent.

CD31 expression was seen in a percentage of all tumors except for granular cell tumors, intradermal Spitz nevi, and epithelioid cell histiocytomas (26 [24%] of 108 Image 1). All nine cases of angiosarcoma expressed this marker, but if they and cases of angiolymphoid hyperplasia with eosinophilia are excluded, staining is seen in 19 (20%) of 95 nonvascular tumors. Eight (8%) nonvascular cases strongly expressed and 11 (13%) cases weakly expressed CD31. In eight (7%) vascular and nonvascular cases, intratumoral histiocytes rather than lesional cells were positive.

Intratumoral histiocytes and lesional cells were distinguished from each other by comparison with the corresponding H&E-stained sections, as previously described. While intrallesional histiocytes typically stain weakly and lesional cells stain strongly, some lesional cells in this series also stained weakly.

Outside of angiosarcomas and cases of angiolymphoid hyperplasia with eosinophilia, CD34 and factor VIII did not mark any of the samples within our set. In cases where CD163 and CD31 markers were used together, they colocalized in three cases of atypical fibroxanthoma, three cases of XG, two cases of LCH, one case of reticulohistiocytoma, and one case of xanthoma.
One case of atypical fibroxanthoma was notable for being originally misdiagnosed as a cutaneous angiosarcoma. The pleomorphic atypical cells of the initial partial biopsy specimen were admixed with areas of angio genesis and extensive erythrocyte extravasation. Although CD31 only weakly highlighted its intralesional histiocytes, no CD34 or factor VIII staining was performed. The final excisional specimen, however, showed more characteristics of atypical fibroxanthoma, and the correct diagnosis was rendered.

### Table 1

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Clone</th>
<th>Pretreatment</th>
<th>Antibody Dilution</th>
<th>Source</th>
<th>Automated Stainer</th>
<th>Staining Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD31</td>
<td>JC70A</td>
<td>Standard retrieval</td>
<td>1:150</td>
<td>Dako, Carpinteria, CA</td>
<td>Ventana BenchMark (Ventana Medical Systems, Tucson, AZ)</td>
<td>Membrane/cytoplasm</td>
</tr>
<tr>
<td>CD34</td>
<td>MY10</td>
<td>Mild retrieval</td>
<td>1:20</td>
<td>BD Biosciences, San Jose, CA</td>
<td>Ventana BenchMark</td>
<td>Membrane</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>F8/86</td>
<td>Standard retrieval</td>
<td>1:40</td>
<td>Dako</td>
<td>Ventana BenchMark</td>
<td>Membrane</td>
</tr>
<tr>
<td>CD163</td>
<td>10D6</td>
<td>Mild retrieval</td>
<td>1:100</td>
<td>Novocastra, Newcastle upon Tyne, UK</td>
<td>Ventana BenchMark</td>
<td>Membrane/cytoplasm</td>
</tr>
</tbody>
</table>

*Mild retrieval incubation for 30 minutes in EDTA (pH 8.0-8.5); standard retrieval incubation for 1 hour in EDTA (pH 8.0-8.5).*

### Table 2

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Cases</th>
<th>Age Range</th>
<th>Male-to-Female Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthogranuloma</td>
<td>12</td>
<td>9 wk to 42 y</td>
<td>8:4</td>
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<tr>
<td>Atypical fibroxanthoma</td>
<td>29</td>
<td>45 to 92 y</td>
<td>25:4</td>
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<tr>
<td>Reticulohistiocytoma</td>
<td>8</td>
<td>27 to 54 y</td>
<td>4:4</td>
</tr>
<tr>
<td>Epithelioid cell histiocytoma</td>
<td>8</td>
<td>18 to 75 y</td>
<td>3:5</td>
</tr>
<tr>
<td>Langerhans cell histiocytosis</td>
<td>9</td>
<td>1 d to 51 y</td>
<td>5:4</td>
</tr>
<tr>
<td>Xanthoma</td>
<td>11</td>
<td>11 mo to 71 y</td>
<td>4:7</td>
</tr>
<tr>
<td>Angiosarcoma</td>
<td>9</td>
<td>59 to 86 y</td>
<td>6:3</td>
</tr>
<tr>
<td>Granular cell tumor</td>
<td>9</td>
<td>14 to 101 y</td>
<td>5:4</td>
</tr>
<tr>
<td>Angiolymphoid hyperplasia with eosinophilia</td>
<td>4</td>
<td>39 to 58 y</td>
<td>2:2</td>
</tr>
<tr>
<td>Intradermal Spitz nevus</td>
<td>9</td>
<td>6 to 83 y</td>
<td>3:6</td>
</tr>
</tbody>
</table>

### Table 3

|CD31, CD34, CD163, and Factor VIII Staining in Cutaneous Histiocytomas, Histiocyte-Rich Tumors, Histiocytoma Mimics, and Angiosarcomas*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Total No. of Cases</th>
<th>CD31, No. (%)</th>
<th>CD31+, No. (%)</th>
<th>P, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesional Cells</td>
<td>Intratumoral Histiocytes</td>
<td>CD163</td>
<td>CD34</td>
<td>Factor VIII</td>
</tr>
<tr>
<td>Xanthogranuloma</td>
<td>12</td>
<td>2 (17)</td>
<td>1 (8)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>Atypical fibroxanthoma</td>
<td>29</td>
<td>4 (14)</td>
<td>6 (21)</td>
<td>10 (34)</td>
</tr>
<tr>
<td>Reticulohistiocytoma</td>
<td>8</td>
<td>1 (13)</td>
<td>0</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Epithelioid cell histiocytoma</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Langerhans cell histiocytosis</td>
<td>9</td>
<td>3 (33)</td>
<td>1 (11)</td>
<td>4 (44)</td>
</tr>
<tr>
<td>Xanthoma</td>
<td>11</td>
<td>1 (9)</td>
<td>0</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Angiosarcoma</td>
<td>9</td>
<td>0</td>
<td>9 (100)</td>
<td>9 (100)</td>
</tr>
<tr>
<td>Granular cell tumor</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Angiolymphoid hyperplasia with eosinophilia</td>
<td>4</td>
<td>1 (25)</td>
<td>3 (75)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>Intradermal Spitz nevus</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*In situations where all cases tested were negative but not all cases were tested, the number tested appears as a denominator P, total number and percentage of cases showing either strong or weak positivity; SP, strongly positive defined as intense staining in at least 20% of cells; WP, weakly positive defined as intense staining of 5% to 19% of cells or faint to moderate staining in at least 20% of cells.

**Image 2.** The pleomorphic atypical cells of the initial partial biopsy specimen were admixed with areas of angiogenesis and extensive erythrocyte extravasation. Although CD31 only weakly highlighted its intralesional histiocytes, no CD34 or factor VIII staining was performed. The final excisional specimen, however, showed more characteristics of atypical fibroxanthoma, and the correct diagnosis was rendered.

### Discussion

CD31, also known as PECAM-1, is a transmembrane glycoprotein whose gene rests on chromosome 17. It is expressed in a variety of different cell types, of which the most relevant for skin include endothelial cells and histiocytes. Little has been published in general about the expression of CD31 in cutaneous histiocytomas, possibly because expression of CD31 in histiocytes in general has not been well documented in the surgical pathology literature.
A. Xanthogranuloma. A dense infiltrate of large epithelioid cells, foam cells, and Touton-type giant cells occupies the dermis. The epidermis is not involved (H&E, ×4).

B. High-power view shows a proliferation of large epithelioid cells with prominent nucleoli and vesicular chromatin arranged in a focal storiform pattern. Small vessels are seen as well as scattered lymphocytes (H&E, ×40).

C. The epithelioid cells express CD31 (×40).

D. Reticulohistiocytoma. A dense infiltrate of lymphocytes and large epithelioid cells occupies the dermis (H&E, ×4).

E. Higher power view shows the epithelioid cells of reticulohistiocytoma to be large with vesicular chromatin, prominent nucleoli, and “ground-glass” cytoplasm. Touton-type cells and foam cells are absent, distinguishing this tumor from xanthogranulomas (H&E, ×40).
is known to be important as an adhesion molecule, modulating endothelial cell-to-cell contact in angiogenesis and leukocyte-endothelial adhesion in inflammatory migration and homing.\textsuperscript{11,12} In addition, CD31 has been documented on macrophages, where it may be playing the role of an adhesion molecule and aiding in tissue migration.\textsuperscript{13,14} Not surprisingly, all vascular tumors in our study expressed CD31, and most expressed CD34 and/or factor VIII.

One of the important aspects of our study is the finding that CD31 is expressed in atypical fibroxanthomas (34% of our cases). In particular, a hemorrhagic, heavily vascularized partial biopsy specimen of a case of atypical fibroxanthoma was initially misdiagnosed as angiosarcoma; excision of the entire tumor revealed the true diagnosis. Our study shows that CD34 and factor VIII do not stain atypical fibroxanthomas but are expressed in angiosarcomas—a finding supported by several recent studies regarding the potential pitfalls in diagnosing atypical fibroxanthomas.\textsuperscript{15-23} The addition of other vascular markers such as CD34, factor VIII, Fli-1,\textsuperscript{15} and/or ERG\textsuperscript{24} is therefore necessary to distinguish between angiosarcomas and atypical fibroxanthomas since CD31 alone is not specific enough. Interestingly, it is clear from comparisons between H&E-stained and CD31-stained sections that, at least in part, some of the staining seen with CD31 is of intratumoral histiocytes. Comparison with CD163 staining helps to confirm this finding.
CD31 staining, however, is not confined to angiosarcomas and atypical fibroxanthomas but is also seen in XGs, xanthomas, reticulohistiocytomas, and cases of LCH. In a series of cutaneous and intramuscular juvenile XGs, weak CD31 staining was seen in a subset of cells in seven (54%) of 13 cases, primarily limited to areas of histiocytic differentiation. This is similar to what we found in our own study, but in at least one of our cases, strong staining was seen. Weak and partial staining is not surprising in XGs, since they are thought to be of histiocytic origin and typically stain strongly with CD163. Likewise, a rare case of xanthoma stained with CD31, and xanthomas are on a histologic continuum with XGs. Most of our xanthoma and XG cases stained with CD163 but not CD31, which may mean that CD31 is not ubiquitously expressed on macrophages—its expression may instead depend on the need to achieve cellular migration.

Likewise, reticulohistiocytomas were rarely marked with CD31, which is similar to the findings of Miettinen and Fetsch, who found that some large epithelioid histiocytes in four (36%) of 11 cases marked with CD31. This is again unusual in comparison to the ubiquitous expression pattern of CD163 in all reticulohistiocytomas. In contrast, all cases of epithelioid cell histiocytoma, a histologic mimic of reticulohistiocytoma, were negative for CD31 in our study. These tumors were similarly negative for CD163, a result supported by prior literature. Taken together, these findings suggest that unlike reticulohistiocytomas, the lesional cells of epithelioid cell histiocytomas are not histiocytes but
probably epithelioid fibroblasts. A tumor with epithelioid histiocytes that expresses both CD31 and CD163 is therefore more likely to be a reticulohistiocytoma or Touton cell-poor XG or xanthoma rather than an epithelioid cell histiocytoma. Furthermore, the term solitary epithelioid histiocytoma, suggested by Miettinen and Fetsch as a replacement for reticulohistiocytoma, may be somewhat misleading since epithelioid cell histiocytomas do not express either CD31 or CD163, unlike reticulohistiocytomas.

Almost half of the cases of LCH were marked with CD31 (four [44%] of nine), one strongly and three weakly, which is similar to the staining pattern of CD163. Unlike the other categories of true histiocytic neoplasms, intratumoral histiocytes were marked in two cases and lesional cells in the other two. Slone et al examined the presence of CD31 in 10 cases of LCH and found expression in all, a nearly 2-fold difference compared with our own study. They did not, however, find CD31 staining in normal intraepidermal Langerhans cells. While one explanation could be gain of expression of CD31 in the transformation of normal Langerhans cells into neoplastic ones, another could be gain of expression of CD31 in proliferating rather than resting Langerhans cells. Regardless, CD31 could again be playing a migratory role in proliferating Langerhans cells and may be upregulated even in reactive processes, such as arthropod bites, that have an abundance of Langerhans cells.

Granular cell tumors and intradermal Spitz nevi, which are also mimics of cutaneous histiocytomas, were negative
for both CD31 and CD163. These entities would show morphologic overlap with LCH if eosinophils were scarce instead of plentiful, since they all strongly express S100. Therefore, a tumor that expresses both CD31 and S100 is most likely to be LCH, rather than a granular cell tumor or intradermal Spitz nevus.

In conclusion, we have tested CD31 in a wide range of cutaneous histiocytic tumors and histiocytic mimics and found that CD31 is indeed expressed in a small subset of cases. The addition of CD34, factor VIII, and other vascular markers may help avoid the misdiagnosis of angiosarcoma, especially in malignant-appearing tumors such as atypical fibroxanthoma. Comparison with H&E-stained and CD163-stained sections shows that at least in part, expression of CD31 is present within both intratumor histiocytes and lesional cells. The overlap between expression patterns of CD163 and CD31 is not absolute and may reflect the differing roles of these two markers in the cellular biology of histiocytes.

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