Immunostaining for CD31 and CD34 in Kaposi sarcoma

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Abstract

Aims—To evaluate antibodies directed against CD31 (JC70/A) and CD34 (QBEND/10 and anti-HPCA-1) more extensively in Kaposi sarcoma; to assess their value in routine diagnosis; and to compare them with the traditional endothelial cell markers Ulex europaeus agglutinin 1 (UEA-1) and factor VIII related antigen. Methods—Twenty four cases of Kaposi sarcoma were studied retrospectively. All specimens had been fixed in formalin and embedded in paraffin wax. The antibodies were applied using the Streptavidin biotin technique in all cases except for UEA-1, for which an indirect two stage method was used involving peroxidase conjugated anti-ulex as the secondary antibody. Results—Tumours were classified into those showing angiomatoid or lymphangiomatoid elements and spindle cell lesions. Universal labelling of all lesions and virtually all elements within lesions was seen with the anti-CD34 antibodies QBEND/10 and HPCA-1. Labelling of spindle cells was less consistent with JC70/A but both markers were superior to the traditional endothelial cell markers UEA-1 and factor VIII related antigen. Conclusions—These data confirm that Kaposi sarcoma is a tumour of endothelial cell origin. They shed further light on the histogenesis of this complex tumour and demonstrate that immunostaining for CD34 and CD31 can be used as an aid to diagnosis in routinely processed tissue.

Keywords: Kaposi sarcoma, CD31, CD34.

Immunohistochemistry has been used extensively in the study of malignant vascular tumours; firstly, to confirm their endothelial cell origin and, secondly, to aid diagnosis in routine histopathology. However, many of the markers which have been used for research purposes cannot be applied to routinely processed tissue because the antigenic determinants do not survive formalin fixation or embedding in paraffin wax. Thus our own studies using monoclonal antibodies such as PAL-E and EN4,12 while valuable in confirming the endothelial cell nature of Kaposi sarcoma and other malignant vascular tumours, cannot be used for evaluating routinely processed specimens. These restrictions do not apply to the traditional endothelial markers, Ulex europaeus agglutinin 1 (UEA-1) and factor VIII related antigen. However, studies of Kaposi sarcoma and angiosarcoma have produced conflicting results, particularly in relation to factor VIII related antigen.14

Monoclonal antibodies directed against CD34 and CD31 have excited interest in the study of vascular tumours. While they are not endothelial cell specific, they are widely expressed in vascular endothelium, particularly in pathological states. There is also some doubt whether they will label lymphatic endothelium.14 Only limited studies of Kaposi sarcoma have been carried out using antibodies directed against CD34 or CD31 and some of these have used cryostat sections. Our study aimed to evaluate these markers more extensively in Kaposi sarcoma, to assess their value in routine diagnosis and to compare them with the traditional endothelial cell markers UEA-1 and factor VIII related antigen.

Methods

Twenty four cases of Kaposi sarcoma were retrieved from the files of St John’s Dermatopathology department, St Thomas’s Hospital, London, and the Department of Dermatology, University of Innsbruck, Austria. All specimens had been fixed in formalin and embedded in paraffin wax. The endothelial cell markers included UEA-1 (Sigma, Poole, Dorset, UK; diluted 1 in 50) and four monoclonal antibodies: anti-factor VIII related antigen (Dako, High Wycombe, UK; diluted 1 in 40); QBEND/10 (Oxford, Basingstoke, UK; diluted 1 in 50), JC70/A (Dako; diluted 1 in 10); and anti-HPCA-1 (Becton Dickinson, Gosport, UK; diluted 1 in 25). The Streptavidin biotin technique was used in all cases except for UEA-1, for which an indirect two stage method was used involving peroxidase conjugated anti-ulex as the secondary antibody. All tissue sections were blocked for endogenous peroxidase for 10 minutes prior to immunolabelling using a 3% hydrogen peroxide in methanol solution. Sections for factor VIII related antigen and UEA-1 labelling were trypsinised in the normal manner for 15 minutes (Sigma). JC70/A labelling required protease digestion (Sigma Type X bacillus thermoproteolyticus rokko) for 30 minutes. QBEND/10 and HPCA-1 did not require predigestion.

Results

HISTOLOGY

Traditionally, Kaposi sarcomas are divided into patch, plaque or nodular lesions. In our study 13 biopsy specimens were patch or plaque
lesions, and 11 were nodular. However, from a histogenetic point of view it is more informative to concentrate on the different elements within a Kaposi lesion, particularly during the earliest phases of development. At this stage two types of lesion can be seen histologically. Angiomatoid lesions are small foci of vascular proliferation associated with a few connective tissues cells, a variable inflammatory cell infiltrate, and some red cell extravasation. The angiomatoid foci usually contain a central vessel with prominent endothelial cells surrounded by an or two thin-walled vessels containing erythrocytes and resembling small capillaries (fig 1A). The lumina of these small vessels can become dilated so that adjacent lesions are separated only by a thin endothelial cell lining, the so-called “back to back” capillaries.

The second type of lesion consists of thin-walled, bloodless vessels dispersed within the connective tissue. These vessels are angulated rather than rounded and resemble small lymphatics. This so called lymphangiomatoid variant is seen commonly, but not exclusively, in HIV related diseases. Occasionally, the lymphatic-like vessels merge to produce a dissection of collagen, similar in appearance to that seen in well differentiated angiosarcomas (fig 1D).

Both lymphangiomatoid and angiomatoid changes may be seen within the same lesion (fig 1C). Of the 13 patch/plaque stage lesions studied, two showed lymphangiomatoid changes only, four showed angiomatoid changes only, and seven showed a mixed pattern. Areas of spindle cell proliferation are sometimes seen in these early lesions, particularly in relation to angiomatoid foci (fig 1A).

<table>
<thead>
<tr>
<th>Immunochemical labelling profile of five monoclonal antibodies in 24 cases of Kaposi sarcoma</th>
<th>Factor VIII related antigen</th>
<th>URA-1</th>
<th>Jc70A</th>
<th>QBEND10</th>
<th>HPCA-1</th>
</tr>
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<tbody>
<tr>
<td>Lymphangiomatoid (n=9)</td>
<td>(R)</td>
<td>(R)</td>
<td>(R)</td>
<td>(R)</td>
<td>(R)</td>
</tr>
<tr>
<td>Reactivity (R) number (N)</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>Angiomatoid (n=11)</td>
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<td>(R)</td>
<td>(R)</td>
<td>(R)</td>
<td>(R)</td>
</tr>
<tr>
<td>Central vessels</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Capsular</td>
<td>N</td>
<td>11</td>
<td>11</td>
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<tr>
<td>Early spindle cells (n=6)</td>
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<td>(R)</td>
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<td>(R)</td>
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<tr>
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<td>(R)</td>
<td>(R)</td>
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<td>+</td>
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<tr>
<td>Spindle cell areas</td>
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</table>

Figure 1. (A) Patch stage lesion showing angiomatoid foci with early spindle cell formation (original magnification, ×10). (B) High power of angiomatoid focus (original magnification, ×40). (C) Plaque stage lesion showing a mixed pattern consisting of angiomatoid foci and lymphatic slits in the surrounding connective tissue (original magnification, ×25). (D) Plaque stage lesion showing lymphangiomatoid changes which have merged to produce an angiosarcoma-like appearance (original magnification, ×10).
In this study six of the 13 early lesions showed spindle cell changes. Eventually, however, the different foci expand and merge to form a network of tissue containing a mixture of vascular and spindle cell elements. In some areas numerous small capillaries are cut in cross-section to give an appearance which has been likened to a sieve (fig 2A). In other areas sheets of spindle cells separated by slit-like spaces containing erythrocytes are evident. These are the histological features classically associated with nodular lesions of Kaposi sarcoma. In later lesions spindle cells may predominate with sieve-like areas absent or confined to the periphery of the nodule.

IMMUNOCYTOCHEMISTRY
The staining profile seen with the different elements in the 24 lesions of Kaposi sarcoma studied is summarised in the table. Figures 2 to 5 illustrate the important findings.

Factor VIII related antigen was consistently positive only in the angiomatoid foci. In addition to staining of the central vessels and surrounding capillaries, there was also diffusion of factor VIII related antigen leading to a blush effect and non-specific staining of the surrounding connective tissue cells (fig 3A). Similar foci were seen within nodular lesions, but factor VIII related antigen did not stain lymphangiomatoid lesions, sieve-like areas, or spindle cells (fig 4A).

Staining with UEA-1 was also largely negative. Apart from central vessels within angiomatoid foci there were no elements consistently stained by this marker. Only one nodular lesion showed positivity in sieve-like and spindle cell areas. However, staining of red cells within nodular lesions often gave a false
appearance of positivity (fig 2C). A few capillaries in angiomatoid foci and in two of nine lymphangiomatoid lesions stained with UEA-1 (fig 4B).

By contrast, the CD34 antibodies provided exceptionally consistent labelling of all elements within the lesions of Kaposi sarcoma. The luminal surfaces of all vascular elements were particularly clearly delineated by these markers which often permitted the appreciation of vessels not apparent on haematoxylin and eosin stained sections. There were no sig-
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Different epitopes on CD34. Our findings, however, do not support this hypothesis in that similar labelling of Kaposi lesions was found with both monoclonal antibodies.

Staining with the pan endothelial cell marker UEA-1 was less reliable in Kaposi sarcoma than either CD34 or CD31. Only one of 11 nodular lesions and two of nine lymphoangiomatoid lesions labelled with UEA-1. By contrast, UEA-1 is a reliable marker for angiosarcoma in formalin fixed tissue. In a previous study it labelled 18 of 19 cases in both formalin and PLP (periodate lysine paraformaldehyde) fixed tissue.

Our findings demonstrate that CD34 is the best marker for labelling Kaposi sarcoma lesions in routinely fixed tissue and shed some light on the histogenesis of Kaposi sarcoma. Thus, the spindle cells seen in early patch and plaque stage lesions are CD34 positive in all cases and CD34 positive in virtually all cases. By contrast, in nodular lesions of Kaposi sarcoma the majority of spindle cells are still CD34 positive but a significant minority are now CD34 negative. In late lesions where spindle cells predominate, CD34 labelling also becomes more variable. This provides evidence of de-differentiation and demonstrates that loss of vascular markers is important within spindle cell lesions.

CD34 is a cell surface protein coded for by the CD34 gene on chromosome 1q32. It is expressed by human haematopoietic cells of both the myeloid and lymphoid series as well as endothelial cells. It may have a role in regulating early events in blood cell differentiation or it may function as an adhesion molecule in both endothelial cells and haematopoietic progenitor cells. CD34 is said to be present on normal venular but not lymphatic endothelium, and has been reported as negative in lymphangiomas. If so, this would strongly favour a vascular origin for the spindle cell elements in Kaposi sarcoma. However, Ramani et al have reported that five of eight cases of lymphangioma were focally positive with QBEND/10 and it is possible that adhesion molecules are expressed more strongly in pathological states. In our own studies some sections showed positive labelling of lymphangiomatoid elements with negative labelling of adjacent "normal" lymphatics. Similar considerations apply to CD31. Again, CD31 is thought to label vascular rather than lymphatic endothelium and is reportedly absent in lymphangiomas and lymphoepithelial cysts. However, Nickoloff found CD31 labelling on both vascular and lymphatic epithelium using cryostat sections, and in the present study some normal lymphatics (that is, those containing small valves) labelled with CD31.

It would seem therefore that in Kaposi sarcoma CD34 and CD31 markers do not help to distinguish between cells derived from lymphatic or vascular endothelium. Rather it suggests that the immunophenotype of the endothelial derived cells in Kaposi sarcoma are unlike those found in normal tissue. Rather than interpreting the immunocytochemical findings in terms of "derivation from", it might be more appropriate to regard
the endothelial cells of Kaposi sarcoma as pathological tissue which has differentiated away from normal endothelium. Certain elements such as the angiomatoid foci still resemble vascular tissue, but by the time spindle cells have come to predominate it is no longer possible to relate endothelial cells to their normal counterparts. In addition, the multifocal nature of Kaposi sarcoma indicates that more than one type of endothelial cell could be involved in its histogenesis.