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Immunohistochemical detection of human herpes virus-8 latent nuclear antigen-1 is useful in the diagnosis of Kaposi sarcoma

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Kaposi sarcoma is a low-grade vascular neoplasm that has been shown by molecular analysis to uniformly express the latent nuclear antigen-1 of human herpes virus 8. Differentiating Kaposi sarcoma from other benign or malignant vascular tumors, as well as other nonvascular spindle cell soft-tissue neoplasms, can be challenging. Thus, detection of human herpes virus 8 in fixed tissues would be diagnostically useful. Recently, a monoclonal antibody to human herpes virus 8 latent nuclear antigen-1 has become commercially available for immunohistochemical analysis. We sought to study the sensitivity and specificity of this antibody in the detection of human herpes virus 8 latent nuclear antigen-1 in Kaposi sarcoma. Fixed, paraffin-embedded tissue sections from 21 cases of Kaposi sarcoma, nine cases of spindle cell hemangioma, five cases of cutaneous angiosarcoma, five cases of dermatofibrosarcoma protuberans, one case of vascular transformation of a lymph node, four cases of pilar leiomyoma, four cases of stasis dermatitis, four cases of pyogenic granuloma, and three cases of spindled melanoma were examined immunohistochemically using the rat monoclonal antibody to human herpes virus 8 latent nuclear antigen-1, open reading frame-73 (Advanced Biotechnologies Inc.). Tissue sections were stained with automated immunostainers (Ventana) using heat-induced epitope retrieval and a standard DAB detection kit (Ventana) modified to detect rat Ab. Strong, diffuse, nuclear staining in $>10\%$ of tumor cells was considered a positive result. In all, 21/21 cases of Kaposi sarcoma showed strong, diffuse, nuclear staining for human herpes virus 8 latent nuclear antigen-1 (100%), whereas all cases of spindle cell hemangioma, cutaneous angiosarcoma, dermatofibrosarcoma protuberans, vascular transformation of lymph node, pilar leiomyoma, stasis dermatitis, pyogenic granuloma, and spindled melanoma were negative for this antigen. The monoclonal antibody to human herpes virus 8 latent nuclear antigen-1, open reading frame-73, is a highly sensitive and specific marker of human herpes virus 8 infection in paraffin-embedded tissue sections of Kaposi sarcoma. As such, it is an extremely useful tool for differentiating between Kaposi sarcoma and other vascular and nonvascular spindle cell lesions, which do not express human herpes virus 8 latent nuclear antigen-1.
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Kaposi sarcoma is a low-grade vascular neoplasm first described by Kaposi[1] in 1872. Four clinical forms have been described: classic Kaposi sarcoma is seen in males of Mediterranean or Eastern European origin. African (endemic) Kaposi sarcoma occurs in younger adults and children in Central Africa. Epidemic (HIV-associated) Kaposi sarcoma is found in immunosuppressed patients with human immunodeficiency virus infection. Kaposi sarcoma associated with immunosuppressive therapy is found in patients treated for transplant rejection. In 1994, Chang *et al* discovered a herpes-like virus in the Kaposi sarcoma cells of a patient with AIDS.[2] The virus is now most widely referred to as Kaposi's sarcoma-associated herpes virus (KSHV) or human herpes virus 8 (HHV-8).[3] It has subsequently been shown to be present in essentially all cases of Kaposi sarcoma[4] as well as other conditions including primary effusion lymphoma,[5–7] some cases of multicentric Castleman's disease,[7–10] reactive angioendotheliomatosis,[11] and in the recently described plasmablastic lymphoproliferative disorders arising in the setting of multicentric Castleman's disease.[12]

Distinguishing Kaposi sarcoma from other benign or malignant vascular tumors, as well as other nonvascular spindle cell soft-tissue neoplasms, can

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be challenging. The differential diagnosis may include cutaneous angiosarcoma, spindle cell hemangioma, dermatofibrosarcoma protuberans, vascular transformation of lymph nodes, pilar leiomyoma, stasis dermatitis, pyogenic granuloma, and spindled melanoma among others. Thus, immunohistochemical detection of HHV-8 in fixed tissues would be diagnostically useful, enabling one to differentiate Kaposi sarcoma from these entities. Recently, a monoclonal antibody to HHV-8 latent nuclear antigen-1 (LNA-1), open reading frame-73 (ORF-73), has become commercially available, which is suitable for immunohistochemistry in fixed tissues.

HHV-8 LNA-1 is a protein encoded for by ORF-73 of the virus' genome. The protein is expressed predominantly during viral latency and appears to play a role in viral integration into the host genome.[13,14] It also interferes in apoptosis via interactions with p53.[15] Antibodies to LNA-1 have been used in formalin-fixed, paraffin-embedded tissues previously.[9,11,12,16,17] At the time of this writing, we know of no study evaluating the utility of HHV-8 LNA-1, ORF-73 antibodies in distinguishing Kaposi sarcoma from other histologically similar vascular and nonvascular spindle cell neoplasms. For this reason, we sought to study the sensitivity and specificity of this antibody in the detection of HHV-8 LNA-1 in Kaposi sarcoma.

Materials and methods

In total, 21 cases of Kaposi sarcoma (Table 1), nine cases of spindle cell hemangioma, five cases of cutaneous angiosarcoma from four patients, five cases of dermatofibrosarcoma protuberans, one case of vascular transformation of a lymph node, four cases of pilar leiomyoma, four cases of stasis dermatitis, four cases of pyogenic granuloma, and three cases of spindled melanoma were studied. The H&E sections of each case were examined and the diagnoses confirmed by a soft-tissue pathologist (JRG). Fixed, paraffin-embedded tissue sections were then examined immunohistochemically using the rat monoclonal antibody to HHV-8 LNA-1, ORF-73 (Advanced Biotechnologies Inc.). Tissue sections were stained with an automated immunostainer (Ventana ES) using heat-induced epitope retrieval and standard DAB detection kit (Ventana) modified to detect rat Ab (three drops rabbit normal serum concentrate, one drop biotinylated rabbit, anti-rat secondary antibody (Vector Laboratories, Burlingame, CA, USA) for every 10 ml of biotinylated immunoglobulin from the standard DAB detection kit). Primary antibody dilution was 1:1000 with an incubation time of 32 min. Antigen retrieval was achieved with a 15-min treatment in a microwave pressure cooker with citrate buffer, followed by a 15-min cool down. Strong, diffuse, nuclear staining in $>10\%$ of the tumor cells was considered a positive result. A cell block of the primary effusion lymphoma cell line BC-3 and a case of Kaposi sarcoma were used as positive controls.

Table 1 Characteristics of Kaposi sarcoma lesions

Case number	*Site*	*Stage*	*Age*	*Sex*
1	Skin, left arm	Patch/plaque	77	M
2	Skin, thigh	Patch/plaque	50	M
3	Skin, right foot	Patch/plaque	46	M
4	Skin, chest	Patch/plaque	51	M
5	Skin, back	Patch/plaque	63	M
6	Skin, shoulder	Patch/plaque	44	M
7	Skin, shoulder	Patch/plaque	47	M
8	Skin, flank	Patch/plaque	59	M
9	Skin, arm	Patch/plaque	36	M
10	Skin, foot	Patch/plaque	39	M
11	Skin, forehead	Nodular	46	M
12	Skin, ear	Nodular	54	M
13	Skin, foot	Nodular	87	M
14	Lymph node	NA	42	M
15	Trachea	NA	38	M
16	Lung	NA	39	M
17	Stomach	NA	41	M
18	Stomach	NA	59	M
19	Duodenum	NA	43	M
20	Rectum	NA	46	M
21	Soft tissues, leg	NA	83	M

NA: not applicable, M: Male.

Results

Characteristic histologic features of Kaposi sarcoma included spindle-shaped tumor cells surrounding hyperemic vascular slits, often accompanied by extravasated erythrocytes, hemosiderin, and fibrosis. The sites of the 21 Kaposi sarcoma specimens are summarized in Table 1. Similar to Kaposi sarcoma, cases of spindle cell hemangioma were composed of bland spindle cell proliferations between vascular lumens with extravasated erythrocytes. However, unlike Kaposi sarcoma, vacuolated cells were sometimes noted lining lumens, as well as epithelioid endothelial cells. Five cutaneous angiosarcomas, two spindled, one epithelioid, and two mixed, were studied from four patients. Infiltrating, anastomosing vascular channels lined by numerous plump spindled-to-epithelioid cells with large hyperchromatic nuclei characterized these cases. Infiltrative lesions with bland spindle cells in a tight, storiform pattern, were the rule for cases of dermatofibrosarcoma protuberans. The lymph node with vascular transformation demonstrated conversion of nodal sinuses into numerous capillary-like spaces containing some erythrocytes. Pilar leiomyomas were composed of nodular, dermal aggregates of poorly circumscribed, intersecting fascicles of eosinophilic spindle cells with plump, cigar-shaped nuclei. The cases of stasis dermatitis all had a superficial dermal vascular proliferation within a background of dermal fibrosis, perivascular

lymphohistiocytic infiltrates, extravasated erythrocytes, and hemosiderophages. There was variable acanthosis and hyperkeratosis. All pyogenic granulomas were nodular proliferations of small capillaries with epidermal ulceration, resembling granulation tissue. Finally, the spindled melanomas had spindled cells with highly atypical nuclei and scant cytoplasm embedded in a fibrotic dermal stroma; cellularity varied.

The results of the immunohistochemical analysis are summarized in Table 2. All 21 Kaposi cases showed strong, nuclear staining for HHV-8 (100%). Cutaneous Kaposi sarcoma cases included 10 patch/plaque and three nodular lesions. Nodular lesions generally had more positive spindle cells compared to patch/plaque stage lesions. Patch/plaque stage lesions demonstrated staining in 10% of the nuclei of spindled/fusiform cells in one case, 20% in four cases, 40% in two cases, 50% in two cases, and 75% in one case (Figure 1a). Nodular stage lesions had 40% positive nuclei in one case, and 50% positive nuclei in two cases (Figure 1b). Non-cutaneous Kaposi sarcoma cases included one mucosal biopsy specimen each from the trachea, duodenum, and rectum, two biopsy specimens from the stomach, an endobronchial biopsy of the lung, and a resection specimen from the soft tissues of the leg. Staining was seen in 20 and 40% of nuclei in the two cases of gastric Kaposi sarcoma, 50% of nuclei in the tracheal, lung, and rectal cases, 75% of nuclei in the duodenal case, and 90% of nuclei in the soft-tissue Kaposi sarcoma (Figure 1c). Importantly, normal vascular endothelial cells were negative in all cases of Kaposi sarcoma. The spindle cell hemangiomas (9), cutaneous angiosarcomas (5), dermatofibrosarcoma protuberans (5), the vascular transformation of a lymph node case (1), pilar leiomyomas (4), cases of stasis dermatitis (4), pyogenic granulomas (4), and spindled melanomas (3) were all negative for this antigen (Figure 1d, Table 2). Of note was the occasional light background staining of eccrine structures and keratinocytes in the skin. (After performance of this study, our clinical laboratory switched from the Ventana ES to the Ventana Benchmark automated immunostainer. The new protocol has eliminated background staining.)

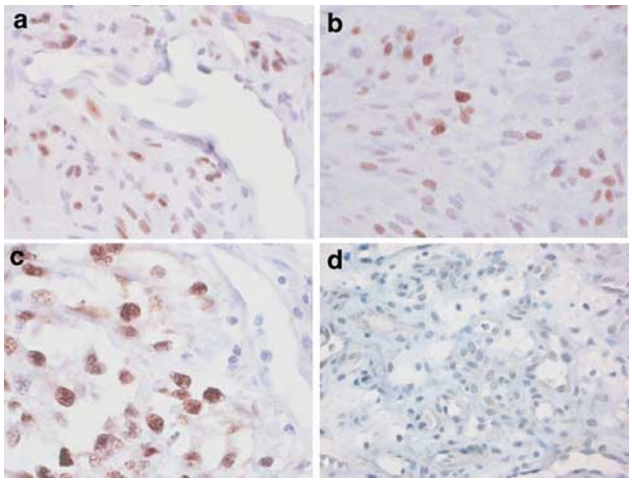

Figure 1 Representative high-power views of immunohistochemical staining for HHV-8 LNA-1 in (**a**), cutaneous patch/plaque Kaposi sarcoma, (**b**) cutaneous nodular Kaposi sarcoma; and (**c**), soft-tissue Kaposi sarcoma. Note that all normal vascular endothelial cells are negative for HHV-8 LNA-1. (**d**) Representative case demonstrating the absence of reactivity for HHV-8 LNA-1 in a case of pyogenic granuloma.

Discussion

In their original paper, Chang *et al*[2] used representational difference analysis (RDA) to analyze 27 cases of AIDS-associated Kaposi sarcoma and 142 non-Kaposi sarcoma cases. They discovered unique DNA sequences in 90% of AIDS-associated Kaposi sarcoma.[2] Using polymerase chain reaction (PCR), Moore *et al*[4] studied 11 cases of AIDS-related, six cases of classic, and four cases of non-HIV-associated Kaposi sarcoma, along with 21 negative controls for the presence of these sequences. PCR product was found in 20 of 21 (95%) tissue samples from the patients with Kaposi sarcoma and only one of 21 control samples (5%). Subsequently, these sequences were found by Moore *et al*[18] to belong to a new human herpes virus, now designated HHV-8.

Table 2 Immunostaining results summary

Tumor type	*HHV-8 positive*	*HHV-8 negative*	*Total*	*Percent*
Kaposi's sarcoma	21	0	21	100
Spindle cell hemangioma	0	9	9	0
Angiosarcoma	0	5	5	0
Dermatofibrosarcoma protuberans	0	5	5	0
Vascular transformation of lymph node	0	1	1	0
Pilar leiomyoma	0	4	4	0
Stasis dermatitis	0	4	4	0
Pyogenic granuloma	0	4	4	0
Spindled melanoma	0	3	3	0
			56	

Distinguishing Kaposi sarcoma from other benign or malignant vascular tumors as well as other nonvascular spindle cell soft-tissue neoplasms may, on occasion, be difficult. The histologic features of these lesions overlap, leading to diagnostic dilemmas. With the discovery of HHV-8 in all forms of Kaposi sarcoma,[4] it became possible to consider virus detection as a potential diagnostic test. Molecular methods have traditionally been used to identify HHV-8 in human tissues. These include PCR amplification,[2,16,19,20] direct *in situ* hybridization,[6,21] *in situ* PCR,[22] and reverse transcriptase (RT) *in situ* PCR.[23] These methods are labor intensive, time consuming, and require highly skilled laboratory personnel. In addition, highly sensitive PCR methodologies have led to a recent controversy. Initial studies using these methods suggested a biological association between HHV-8 and multiple myeloma. However, most experts now believe these to be 'false' positives attributed to overamplification of 'bystander' HHV-8 sequences commonly present in healthy individuals with a low rate of infection.[24–26] Recent commercial availability of a monoclonal antibody to HHV-8 LNA-1 has made cost-effective, tissue-localized identification of HHV-8 in fixed human specimens possible. The reliable detection of HHV-8 in fixed tissues by immunohistochemistry could enable one to differentiate Kaposi sarcoma from other histologically similar entities. For this reason, we sought to study the sensitivity and specificity of this antibody in the detection of HHV-8 LNA-1 in Kaposi sarcoma.

All of our Kaposi sarcoma cases demonstrated strong, nuclear staining for HHV-8 (100%), whereas all cases of spindle cell hemangioma (9), cutaneous angiosarcoma (5), dermatofibrosarcoma protuberans (5), vascular transformation in a lymph node (1), pilar leiomyoma (4), stasis dermatitis (4), pyogenic granuloma (4), and spindled melanoma (3) were negative for this antigen (0%). The uniform expression of HHV-8 in all of our cases of Kaposi sarcoma (100% sensitivity) confirms the observations of previous investigators using other methods.

Antibodies to HHV-8 LNA-1 have been used previously to identify HHV-8 by IHC.[9,11,12,16,17] In a survey of HHV-8+ lesions, Dupin *et al*[9] used an antibody to HHV-8 LNA-1 to study the distribution of cell types latently infected by HHV-8 in patch/plaque and nodular Kaposi sarcoma, multicentric Castleman's disease, and primary effusion lymphoma. These authors studied 14 cases of Kaposi sarcoma and found nuclear staining in all stages of Kaposi sarcoma. They noted fewer positive cells in patch/plaque lesions compared to nodular lesions. No staining was found in normal endothelium. Our results are similar to Dupin *et al*, as we noted a similar pattern of staining with fewer immunoreactive spindle cells noted in patch/plaque lesions compared to nodular lesions. These authors also found LNA-1 expression in cells of other HHV-8 positive lesions such as multicentric Castleman's disease and primary effusion lymphoma, but no staining was found in samples of multiple myeloma, prostate cancer, or angiosarcoma.[9] Unlike Dupin *et al*, we also studied examples of spindle cell lesions that might enter the differential diagnosis of Kaposi sarcoma (eg dermatofibrosarcoma protuberans), and found them to be uniformly negative for HHV-8 LNA-1. In a study of 13 cases of multicentric Castleman's disease using the same antibody, Du *et al*[12] found that all the 13 (100%) cases contained a majority of plasmablasts positive for HHV-8. Most recently, McMenamin *et al*[11] reported that four of their cases of reactive angioendotheliomatosis stained positive for HHV-8 LNA in lesional cells. Interestingly, two of the four cases arose in immunosuppressed patients. Cool *et al*[17] have also reported HHV-8+ cells both in and around plexiform lesions in a subset of cases of primary pulmonary hypertension. These results may expand the types of vascular lesions in which HHV-8 may be detected.

The high sensitivity and specificity of automated immunohistochemical detection for HHV-8 in Kaposi sarcoma with this antibody make it a reliable and cost-effective means of differentiating Kaposi sarcoma from other vascular and nonvascular spindle cell lesions. Furthermore, since it does not rely on amplification of nucleic acid and allows tissue localization, false-positive results or detection of 'bystander' virus may be less likely. We believe it to be an extremely useful tool to the surgical pathologist confronted with a lesion in which Kaposi sarcoma is a diagnostic consideration.

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