Expression of CD31, CD34, and Factor Ⅷ-related Antigen in Vascular and Spindle Cell Tumors of the Skin

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Background **: The immunohistochemical detection of endothelial differentiation in skin tumors has been hindered by the relative paucity of reliable markers that are applicable to routinely-processed specimens, which are both specific and sensitive as well.**

Objective **: This study was designed to evaluate the utility of CD31 and CD34, newly introduced vascular markers, in the immunohistochemical differentiation of vascular neoplasms from other mesenchymal tumors and to compare their sensitivity and specificity with factor Ⅷ-related antigen (FⅧRA).**

Methods **: Paraffin-embedded specimens of 26 cases of benign and malignant vascular tumors and 25 cases of non-vascular mesenchymal neoplasms of the skin and subcutis were investigated for CD31, CD34, and FⅧRA expression using immunohistochemical methods.**

Results **: CD31-immunoreactivity was observed in all of the 26 vascular lesions. CD34 and FⅧRA were labelled in 23 cases and 16 cases of vascular tumors, respectively. All of the 25 non-vascular mesenchymal tumors were negative for CD31 and FⅧRA. In contrast, positive reactivity for CD34 was seen in 14 cases, among which dermatofibrosarcoma protuberans(2/2), neurofibroma(8/8), neurilemmoma(2/2), and leiomyoma(2/3) were included.**

Conclusion **: Our results indicate that CD31 is a more sensitive and specific vascular marker than CD34 and FⅧRA. A small panel composed of these three antibodies will constitute a comprehensive and reliable method for identifying tumors of vascular origin.**

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Key Words : CD31, CD34, FⅧRA, Vascular tumor

An accurate diagnosis of vascular neoplasms is increasingly dependent upon immunohistochemistry. For immunohistochemical evaluation of vascular neoplasms, Factor Ⅷ-related antigen (FⅧRA) and Ulex europaeus agglutinin-1 (UEA-I) have been widely used as markers for endothelial cells[1-4]. However both these markers have limitations. FⅧRA has proven to be a highly specific marker, but it is reported to be negative in some proliferative states of endothelial cells and in malignant vascular tumors[4-7]. Although UEA-I is a more sensitive marker for vascular differentiation, it is less specific, since positive reactivity may also occur in many epithelial tumors[5,8,9]. These situations have raised many questions about the reliability of these antibodies for pathological investigations and have stimulated a search for better reagents. Re-

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cently, CD31 and CD34 have been shown to have utility as indicators of endothelial differentiation[9-12]

CD31 (also known as platelet endothelial cell adhesion molecule-1〔 PECAM-1〕) is a 130kD transmembrane glycoprotein present in endothelial cells and in a variety of hematopoietic cells including platelets, subsets of lymphocytes and plasma cells, and early hematopoietic stem cells[13]. It is a member of the immunoglobulin gene superfamily consisting of six Ig-related domains. The gene for CD31 is located on the long arm of chromosome 17[13]. The wide distribution of CD31 among vascular cells suggests that it may have several important physiological functions. This has been strengthened by studies which have highlightened roles for this molecule in the recruitment of leukocytes into inflammatory sites and in the process of angiogenesis[13,14].

CD34 is a 110kD heavily glycosylated transmembrane protein which is encoded by a gene on chromosome 1q[10]. It is expressed on hematopoietic progenitor cells in the bone marrow and on endothelial cells in numerous nonhematopoietic organs including the skin[10]. Although the exact function of CD34 is currently unknown, several lines of evidence suggest that CD34 might play a role in cell adhesion or signal transduction[15].

Although CD31 and CD34 have been suggested as markers for vascular tumors, the knowledge of their distribution patterns in different types of cutaneous vascular tumors is incomplete[16]. This study was designed to evaluate the utility of CD31 and CD34 in the immunohistochemical differentiation of vascular neoplasms from other mesenchymal tumors of the skin and compare these results with stains of the FⅧRA.

Table 1. Monoclonal antibodies used in this study

Antibodies	Antigens	Sources	Concentrations
JC70	CD31	Dako	1:40
QBEND10	CD34	Immunotech	1:50
F8/86	FⅧRA	Dako	1:50

MATERIALS AND METHODS

Tissue samples

Twenty six cases of benign and malignant vascular neoplasms and 25 cases of non-vascular mes-

Table 2. Immunohistochemical results in vascular neoplasms

Diagnoses	Number	CD31	CD34	FⅧRA
Pyogenic granuloma	9	9	9	8
Lymphangioma	5	5(3)	4(4)	1(1)
Hemangioma	4	4	4	4
Angiosarcoma	4	4	2*	0
Glomus tumor	3	3	3	3
Kaposi's sarcoma	1	1	1	0
Total	26	26	23	16

( ) ; weakly focal positive
* ; poorly differentiated type

Table 3. Immunohistochemical results in non-vascular mesenchymal tumors

Diagnoses	Number	CD31	CD34	FⅧRA
Dermatofibroma	10	0	0	0
Neurofibroma	8	0	8	0
Leiomyoma	3	0	2	0
Neurilemmoma	2	0	2	0
DFSP	2	0	2	0
Total	25	0	14	0

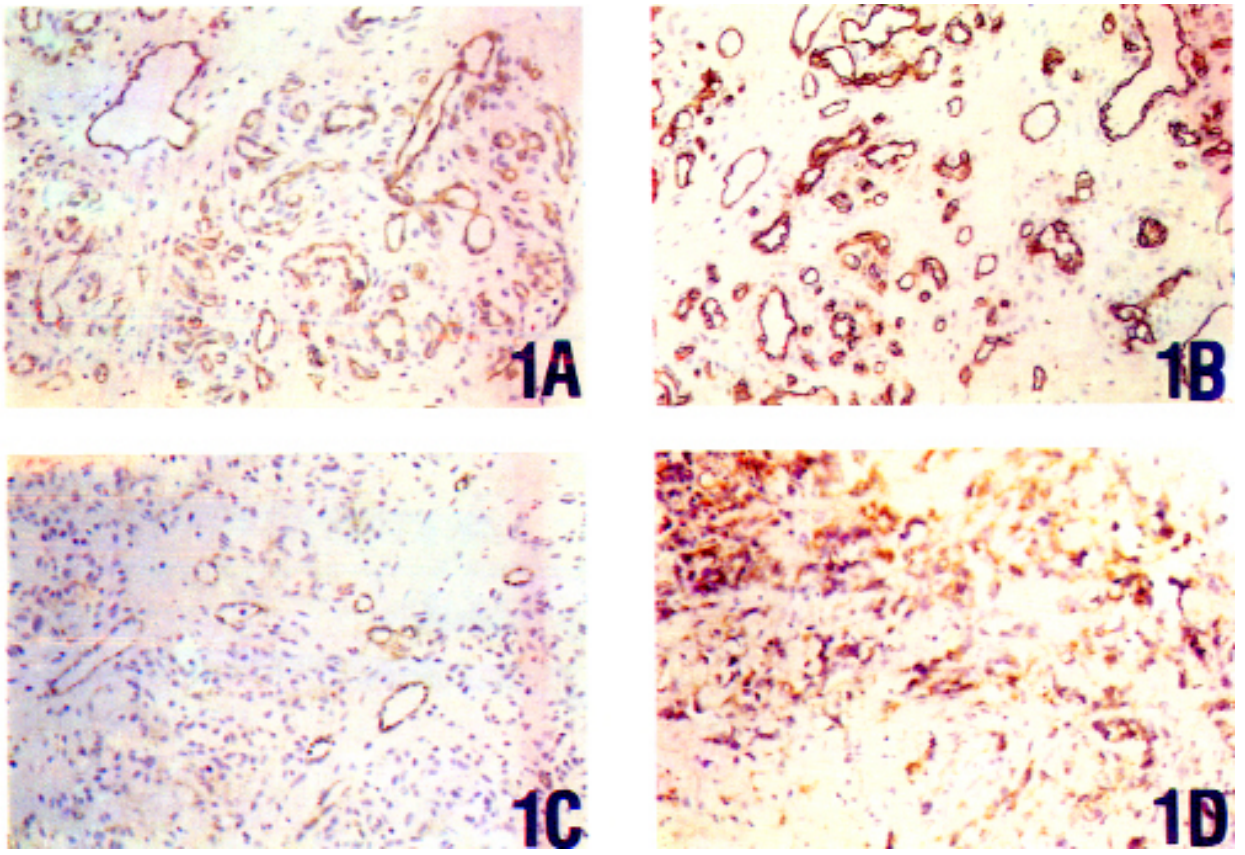

Fig. 1. Immunohistochemical staining for CD31(A), CD34(B), FⅧRA(C) in pyogenic granuloma (×200); diffuse staining for CD31 in poorly differentiated angiosarcoma(D) (×200).

enchymal tumors were studied. Vascular lesions included pyogenic granuloma(9 cases), lymphangioma(5), capillary hemangioma(4), glomus tumor(3), angiosarcoma(4), and Kaposi's sarcoma(1), and non-vascular tumors consisted of dermatofibroma(10), neurofibroma(8), leiomyoma(3), neurilemmoma(2), and dermatofibrosarcoma protuberans (DFSP)(2). Most cases were derived from the files of the department of diagnostic pathology, Samsung Medical Center. Hematoxylin and eosin stained sections of lesions were reviewed and the diagnoses were confirmed. A representative block of formalin-fixed, paraffin-embedded tissue was chosen in each case for immunohistochemical assessment.

Immunoperoxidase staining

Immunohistochemical studies on formalin-fixed, paraffin-embedded tissue sections were performed using the avidin-biotin complex technique. Briefly, 5μm thick sections were cut, deparaffinized in xylene, and rehydrated in ethanol. Endogenous peroxidase activity was blocked by 30 minutes incubation with 3% hydrogen peroxide in absolute methanol. Background staining was minimized by preincubation with normal horse serum(1:20 dilution). Sections to be stained for CD31 and FⅧRA were treated with boiling citrated buffer(pH 6.0) in a microwave for 10 minutes. Sections were then incubated with antibodies against CD31, CD34, and FⅧRA, overnight at 4℃ in moisture chambers. The primary monoclonal antibodies used in our study are listed in Table 1. Staining using the avidin-biotin complex kit (Vector Laboratories, Burlingham, CA, U.S.A.) was done with 3,3-diaminobenzidine tetrahydrochloride (Sigma, St, Louis, MO, U.S.A.) as the chromogen. Sections were counterstained with Mayer's hematoxylin. Both negative and positive controls were used in all cases, with omission of the primary antibodies acting

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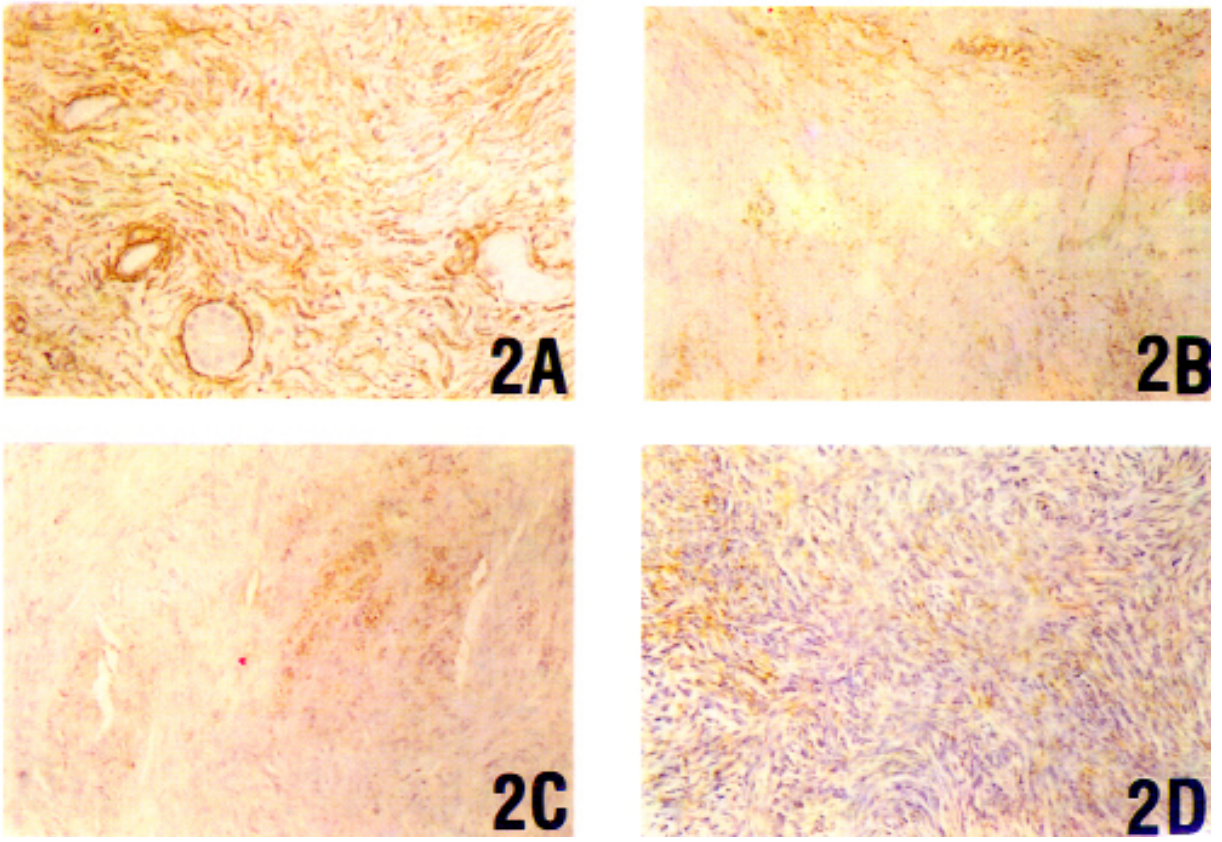

Fig. 2. CD34 expression in nonvascular spindle cell tumors :(A) neurofibroma (×200); (B) neurilemmoma (×100); (C) leiomyoma (×100); (D) DFSP (×200).

as the negative control and the inclusion of normal tissue containing vessels as the positive control.

Assessments

The staining intensity was graded as negative, weak positive, or strong positive. The distribution of positive staining was recorded as focal (less than 50 %) or diffuse (more than 50%).

RESULTS

Normal skin

All the three antigens were expressed on the endothelial cells of the cutaneous vasculature. CD31 and FⅧRA were negative on the epidermis and other connective tissue elements, but CD34 labelled the dendritic interstitial cells, perivascular, perifollicular, and periglandular cells. In general, staining intensity with FⅧRA was much weaker than those with CD31 and CD34.

Vascular tumors

The expression of the 3 endothelial antigens in vascular tumors is summarized in Table 2. Capillary hemangioma and pyogenic granuloma showed diffuse, strong positive reactions with CD31 and CD34. In contrast, FⅧRA showed variable staining patterns; 4 cases with diffuse strong positive reactions, 8 cases with weak focal staining, and the remaining one case with a negative result. CD31 reactivity was strictly limited to the endothelial cells lining mature capillaries(Figure 1A), but CD34 and FⅧRA were also labelled on the adjacent immature cells with weak granular patterns(Figure 1B, 1C).

In cases of Lymphangioma, CD31 staining was seen in all the cases, among which 2 cases showed reactivity on the majority of the endothelial cells lining lymphatic channels, and 3 cases were focally weak positive. CD34 expression was seen in 4 cases with weak and focal reaction. FⅧRA was positive

only in 1 case, in which focal and weak reactivity was observed.

Four cases of angiosarcoma were divided into 2 groups by histological appearances[17] - well differentiated and poorly differentiated. Two cases of well-differentiated angiosarcoma showed positive reaction for both CD31 and CD34. The remaining 2 cases of poorly differentiated tumors, however, were positively labelled only for CD31(Figure 1D), with variable staining intensity. FⅧRA labelling was not detected in any cases.

Kaposi's sarcoma showed positive reactions in angiomatoid areas for CD31 and CD34, but negative with FⅧ RA. Our case was probably the early stage Kaposi's sarcoma with little spindle cell components, so we couldn't observe the reaction pattern on spindle cells.

In glomus tumors, luminal endothelial cells were focally weak positive with all three reagents, but round glomus cells were uniformly negative.

Non-vascular tumors

The distribution of the endothelial antigens in the non-vascular tumors is shown in Table 3. All 25 cases of tumors included in this study failed to stain for CD31 and FⅧRA. All of the 8 cases of neurofibroma showed positive reactions for CD34, of which 4 cases were diffusely strong positive, and the others were focally weak positive(Figure 2A). In addition, two out of 3 leiomyomas showed focally weak immunoreactivity and all of the 2 DFSP gave diffusely strong positive reactions(Figure 2B, 2C). In 2 cases of neurilemmoma, solidly cellular Antoni A area were devoid of CD34-positive cells, whereas in the Antoni B areas, variable proportions of CD-34 positive cells were observed(Figure 2D).

DISCUSSIONS

In this study, we evaluated the specificity and sensitivity of three different endothelial markers in the immunohistochemical identification of neoplastic endothelial cells. This application of immunohistochemistry is not only important in the diagnosis of angiosarcoma and related vascular tumors, but it also has potential in the evaluation of tumor angiogenesis, a central event in the propagation of malignant neoplasms. Endothelial cell markers can also be applied in the documentation of vascular space invasion. FⅧ RA has been commonly used as a marker for vascular endothelial cells for more than a decade[1,2]. But its diagnostic value is limited because of lack of sensitivity and reproducibility[3,4,11,16,17]. The variability of FⅧRA expression on endothelial cells may be related to the maturation status of the cell[18]. This would explain the disappointing results in many vascular tumors, especially malignant ones, which will be at stages of differentiation not recognised by FⅧRA antibodies[18]. In agreement with previous reports, we found that about 70% of benign vascular tumors were positively stained with FⅧRA, but Kaposi's sarcoma, angiosarcoma, and non-vascular mesenchymal neoplasms were uniformly negative. Our results indicate that FⅧ RA may be still used as a specific endothelial marker, even though it is less sensitive than CD34 and CD31.

Both CD31 and CD34 are glycoproteins that are shared between hematopoietic cells and selected endothelia[14,19]. Physiological sharing of these proteins between hematological elements and endothelial cells appears to reflect the close interaction between these cell types in such processes as inflammatory cell margination within blood vessels, transvascular migration of leukocytes, adherence of platelets to vascular walls, and cytokine-promulgated angiogenesis[14,19]. Several reports have demonstrated the potential utility of CD31 and CD34 as markers for endothelial differentiation of vascular neoplasms[9,12,16-18]. In our study, all cases of hemangioma and pyogenic granuloma showed strong diffuse positivity for both CD31 and CD34, which was favorably compared with staining for FⅧRA, broadly in agreement with previous studies.

Lymphangioma showed variable results. The staining intensities of lymphangioma for three reagents were much weaker than those of hemangioma and the distribution was focal except for 2 cases stained with CD31. Therefore, combined application of antibodies against these three antigens may be used as a useful tool in differentiating vascular endothelium from lymphatics.

Recently, Orchard et al.[17] reported that among 19 cases of cutaneous angiosarcoma including 10 cases of poorly differentiated ones, CD31, CD34, and FⅧRA were expressed in 17, 4, and 2 cases, respectively. In our study, all cases of cutaneous angiosarcoma were CD31 positive. In contrast, only two cases were positive for CD34, and immunore-

activity for FVIIIRA was not detected. CD34 reactivity was not observed in 2 cases of poorly differentiated tumors. Our results are in accord with those of Orchard et al., and suggest that CD31 labelling is of diagnostic value at all stages of tumor differentiation. A number of recent reports have highlighted cytokeratin expression in angiosarcoma, most notably in epithelioid forms[17], causing potential difficulties in the differential diagnosis of epithelial derived carcinomas. It appears that cytokeratins 8 and 18 are the most commonly expressed forms[17]. Conversely, squamous cell carcinoma or metastatic adenocarcinoma may demonstrate limited reactivity for CD34[10]. Although CD31 seems to be present in a high number of angiosarcoma, it is absent in the rare cases which show other endothelial markers[20]. Above findings suggest that use of multiple endothelial markers are desirable for differential diagnosis of malignant tumors suspected of endothelial origin.

Previous studies on Kaposi's sarcoma using antibodies against CD31 and CD34 have been limited. Ramani et al.[9] in a study of 40 cases reported positive CD34 labelling of angiomatoid elements with variable staining of spindle cell elements. Parums et al.[11] reported negative CD31 labelling in spindle cells of 4 cases of Kaposi's sarcoma, whereas Nickoloff reported positive labelling on cryostat sections[21]. Jones et al.[22] observed universal labelling of all cases of Kaposi's sarcoma with CD34 and less consistent staining of spindle cells with CD31. These findings contrasted with the results in angiosarcoma where CD31 was found to be a more reliable marker than CD34. Our case revealed positive reaction in angiomatous areas for both markers, but not for FVIIIRA. Lack of spindle cell components prevented us from observing immunoreactivity on these cells.

Nonvascular mesenchymal tumors including neurofibroma, leiomyoma, neurilemmoma, and DFSP were also positively stained with CD34. In normal skin, CD 34 presence has been detected in perivascular/interstitial dendritic cells, predominantly in the reticular dermis, in the perifollicular area of the middle portion of the follicles, and in spindle-shaped cells in the basement membrane zone of the eccrine glands, as well as endothelial cells[23]. Considering such a relatively widespread normal distribution of CD34 in connective tissues, it is not surprising that it can be demonstrated in a number of nonendothelial cell tumors. Such a broad spectrum of CD34 reactivity in different mesenchymal tumors may be a diagnostic pitfall if a single marker approach is used, especially in small biopsies. These findings emphasize the importance of the comprehensive knowledge of the tissue distribution of all antigens, and the use of panels of antibodies to address different possibilities in diagnostic immunohistochemistry.

In conclusion, our results indicate that CD31 is a relatively sensitive and specific marker for endothelial cells compared to CD34 and FVIII RA. Three antibodies against CD31, CD34, and FVIII RA gave similar, but not the same immunostaining of vascular neoplasms. So a small panel composed of these three antibodies will constitute a comprehensive and reliable method for identifying tumors of vascular origin.

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