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Assessment of Diagnostic Utility of anti-CD34 in Soft Tissue Tumors

Hye-Rim Park, M.D., Yong-Koo Park, M.D.*

Department of Pathology, College of Medicine, Hallym University and Kyung Hee University, Seoul, Korea*

We have performed this study to define the usefulness of an anti-human progenitor cell antigen-1(anti-CD34) to distinguish some kinds of soft tissue tumors in formalin-fixed, paraffin-embedded tissues. Sixty three cases of vascular, fibrohistiocytic, neural and other tumors were immunostained for CD34 using the streptavidin-biotin immunoperoxidase method. All of the vascular tumors including hemangiomas, epithelioid hemangioendotheliomas, hemangiopericytomas, and lymphangiomas revealed strong CD34 positivity along the cytoplasmic membranes. Among the fibrohistiocytic lesions, all of five examples of dermatofibrosarcoma protuberans showed diffuse, strong, and linear staining along the cytoplasmic processes. In contrast, none of the benign fibrous histiocytomas and malignant fibrous histiocytomas expressed CD34. CD34-positive cells with delicate dendritic processes could be identified within the normal nerves, neuromas, neurofibromas, and Antoni B areas of neurilemomas. However, all of the malignant peripheral nerve sheath tumors were uniformly negative. In addition, an epithelioid sarcoma and four cases of leiomyosarcoma revealed focal, weak positivity with anti-CD34. In conclusion, this study demonstrated variable anti-CD34 staining pattern of certain fibrohistiocytic, muscle, and neural tumors and confirmed the potential usefulness of anti-CD34 in differentiating fibrous histiocytoma from dermatofibrosarcoma protuberans. It's also helpful to diagnose epithelioid hemangioendothelioma from other epithelioid-type tumors.

Key Words : *CD34, Vascular tumor, Dermatofibrosarcoma protuberans, Neurogenic tumor*

INTRODUCTION

Soft tissue sarcomas are capable of causing diagnostic bewilderment for the surgical pathologist, because of their diversity of differentiation and overlapping histologic appearances. Immunohistochemical studies provide a means whereby this confusion can be resolved in some cases, and electron microscopy also may play a useful role(Wick et al., 1988 ; Angervall and Kindblom, 1993).

The human hematopoietic progenitor cell antigen is known as CD34. It is a single chain transmembrane glycoprotein with a molecular weight of 115 kilodaltons. This antigen is selectively expressed on most hematopoietic colony forming cells from normal human bone marrow and in a significant proportion of

Address for correspondence : *Hye-Rim Park, M.D., Department of Pathology, HanGang Sacred Heart Hospital, 94-200, Yongdungpo-dong, Yongdungpo-gu, Seoul, 150-020, Korea*
Tel : 02-633-9111 ext.1287

acute leukemias(Cohen et al., 1993). It has also been identified in vascular endothelial cells, particularly those engaged in active angiogenesis as well as benign and malignant vascular tumors(Cohen et al., 1993 ; Weiss and Nickoloff, 1993). Most recently, this antigen, or a closely related epitope, has been localized to dendritic cells within the dermis and to slender spindled cells surrounding adnexal structures, the bulge portion of hair shafts, and blood vessels, suggesting that it defines a subset of connective tissue cells that surround or envelope normal structures in skin(Weiss and Nickoloff, 1993).

We did immunostaining using anti-human progenitor cell antigen-1 (anti-CD34) to differentiate some cumbersome soft tissue tumors.

MATERIALS AND METHODS

Tissue samples

We evaluated 63 formalin-fixed, paraffin-embedded tissue specimens for CD34 immunoreactivity. These consisted of vascular, fibroblastic-fibrohistiocytic, neural, and other lesions(Table 1). The formalin-fixed paraffin-embedded tissue samples were retrieved from the files of the Department of Surgical pathology at Hallym University and Kyung Hee University. We reviewed all of the cases and confirmed diagnosis with standard morphologic criteria.

Immunohistochemical studies

The 4 μm sections were stained for CD34 by incubation with the prediluted monoclonal antibody anti-HPCA-1 (anti-CD34, QBEND10 clone, Immunotech, Marseille, France). Antibody binding was made visible by applying the streptavidin-biotin immunoperoxidase method (DAKO LSAB kit, Carpinteria, CA). Negative controls without primary antibody were run in parallel. Staining of endothelium in non-neoplastic vessels served as an endogenous positive control.

Evaluation

The intensity of staining was graded as negative, weakly positive, or strongly positive. The distribution of positive staining was described as focal or diffuse. The pattern of staining was characterized by either linear (membraneous) or cytoplasmic or both.

RESULTS

Vascular lesions

The endothelial cells of all of the hemangiomas (3/3) studied uniformly expressed CD34 antigen and showed diffuse, strongly positive, membraneous staining with anti-CD34(Fig.1). Anti-CD34 showed weak, focal and mainly luminal reaction with endothelial cells lining lymphatic channels of the lymphangiomas (2/2). Both of the two cases of epithelioid hemangioendotheliomas showed weak to moderate CD34 antigen positivity. Staining of intracytoplasmic lumina in the tumor cells was especially evident and the reaction was predominantly granular and cytoplasmic(Fig.2). Two hemangiopericytomas were examined, and the neoplastic hemangiopericytic cells in two cases stained positively for CD34. However, the staining intensity of the neoplastic cells in the hemangiopericyt-

Table 1. Immunohistochemical staining result with anti-CD34 in paraffin sections.

Diagnosis	Cases with CD34 reactivity/ total No. of cases
Vascular tumor	
Lymphangioma	2/2
Hemangioma	3/3
Epithelioid hemangioendothelioma	2/2
Hemangiopericytoma	2/2
Glomus tumor	0/1
Angiofibroma	0/1
Angioleiomyoma	0/1
Fibroblastic and Fibro-histiocytic tumor	
Fibrous histiocytoma	0/4
Juvenile xanthogranuloma	0/2
Giant cell tumor of tendon sheath	0/3
Dermatofibrosarcoma protuberans	5/5
Malignant fibrous histiocytoma	0/3
Fibrosarcoma	0/2
Fibromatosis	0/2
Nodular fasciitis	0/2
Neurogenic tumor	
Neuroma	3/3
Neurofibroma	5/5
Neurilemmoma	3/5
Malignant peripheral nerve sheath tumor	0/5
Others	
Epithelioid sarcoma	1/1
Synovial sarcoma	0/2
Leiomyosarcoma	4/4
Rhabdomyosarcoma	0/2
Liposarcoma	0/1
Total	63 cases

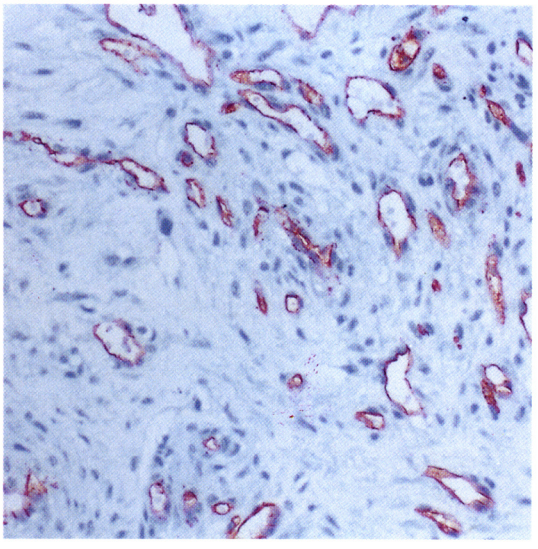

Fig. 1. The endothelial cells of capillary hemangioma showed diffuse, membraneous staining(ABC for CD34, ×200).

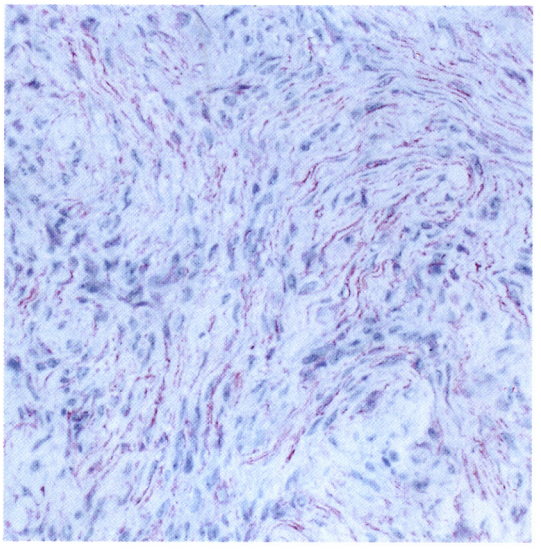

Fig. 3. Dermatofibrosarcoma protuberans showed linear staining pattern along the cytoplasmic processes of tumor cells (ABC for CD34, ×200).

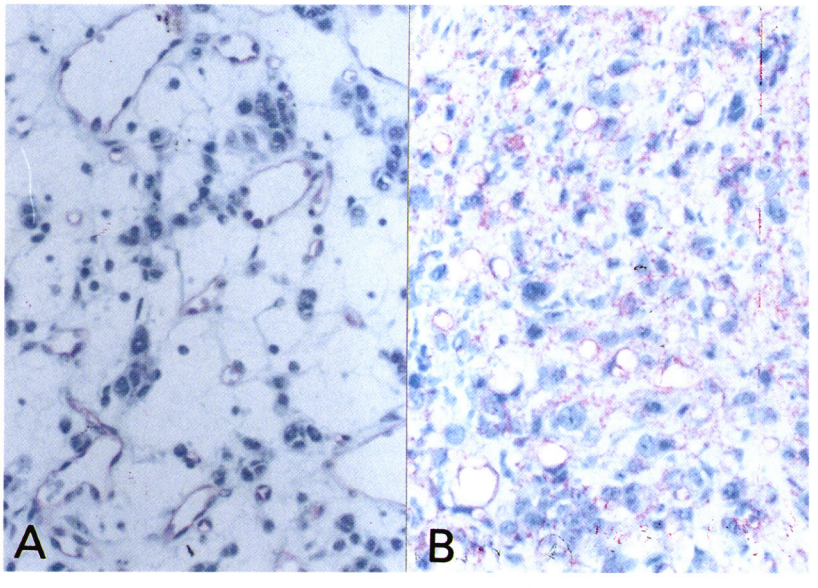

Fig. 2. A&B. Epithelioid hemangioendotheliomas showed weak staining of intracytoplasmic lumina in the tumor cells(ABC for CD34, ×200).

omas was weaker than those of the hemangioendotheliomas. Glomus tumor, angiofibroma, and angioleiomyoma were anti-CD34 negative except for their vascular channels (Table 1).

Fibroblastic and fibrohistiocytic lesions

Six lesions from nodular fasciitis, fibromatosis, and fibrosarcoma did not express anti-CD34.

Of the fibrohistiocytic lesions, all of five examples of dermatofibrosarcoma protuberans showed diffuse, linear staining pattern(Fig.3). The slender cytoplasmic processes of tumor cells were intensely decorated by anti-CD34. In contrast to the dermatofibrosarcoma protuberans, none of the benign fibrous histiocytomas

(0/4) expressed CD34. Juvenile xanthogranulomas (0/2) and giant cell tumors of tendon sheath (0/3) also did not stain with anti-CD34. The spindle cells in malignant fibrous histiocytoma were negative (0/3) (Table 1).

Neural lesions

The neural tumors demonstrated variable expression of CD34 antigen. CD34-positive cells with delicate dendritic processes could be identified within the endoneurium of normal nerve and neuroma. The Schwann cells were negative for anti-CD34. Occasionally CD34-positive cells were also condensed just outside the perineum.

All examples of neurofibroma (5/5) contained a significant but variable portion of membraneous CD34-positive cells(Fig.4). In contrast to neurofibromas, neurilemmomas showed a rather different pattern of immunoreactivity (3/5). The solid cellular Antoni A areas were devoid of CD34-positive cells, whereas in the Antoni B areas, CD34-positive cells were identified with a linear appearance similar to those of neurofibromas(Fig.4). However, all of the malignant peripheral nerve sheath tumors were uniformly negative (0/5) (Table 1).

Others

One case of epithelioid sarcoma revealed focal, weak positive reaction with anti-CD34 in a few scattered tumor cells. All the four leiomyosarcomas revealed focal, weak, and linear staining pattern for anti-CD34. Synovial sarcoma, rhabdomyosarcoma, and liposarcoma were negative for CD34 (Table 1).

DISCUSSION

The expression of CD34 antigen has previously been described on the hematopoietic colony forming cells from normal human bone marrow and acute leukemia, on the endothelial cells of benign and malignant lesions, and on spindle-shaped cells around dermal adnexal structures(Kutzner, 1993). This study, evaluating CD34 antigen immunoreactivity in variable soft tissue tumors, demonstrated variable anti-CD34 staining of certain fibrohistiocytic, muscular, and neural lesions. The authors' investigation also confirmed the potential usefulness of the anti-CD34 to differentiate not only benign and malignant fibrohistiocytic lesions but also dermatofibrosarcoma protuberans.

The monoclonal anti-endothelial antibody QBEND10 is a useful marker in the diagnosis of vascular and lymphatic tumours in formalin-fixed, paraffin-embedded sections(Ramani et al., 1990; Traweek et al., 1991; Kutzner, 1993). Two instances of epithelioid hemangioendothelioma studied were strongly positive for CD34. The positive staining pri-

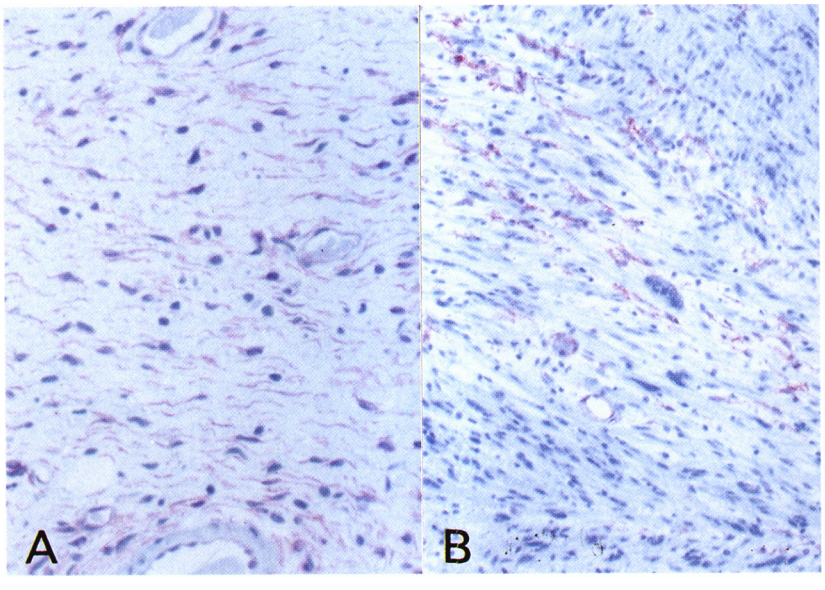

Fig. 4. Neurofibroma(A) and Antoni-B area of neurilemmoma(B) showed linear, wavy CD34-positive cells(ABC for CD34, ×200).

marily was localized to the cell membranes and conspicuous along cytoplasmic lumina. This kind of positive reaction of epithelioid hemangioendothelioma for anti-CD34 can be helpful in differentiating from other epithelioid-type tumors(Ramani et al., 1990; Traweek et al., 1991; Sirgi et al., 1993). Two hemangiopericytomas also stained positively for CD34, but the staining intensity was much less than in hemangioendotheliomas. The presence of low-level CD34 expression in pericytes is acceptable because endothelial cells and pericytes may be derived from a common precursor(Traweek et al., 1991).

Histologic distinction of dermatofibrosarcoma protuberans (DFSP) from fibrous histiocytoma may be difficult. In addition, differential diagnosis is hampered by the lack of appropriate immunohistochemical markers that reliably distinguish dermatofibrosarcoma protuberans from benign and malignant fibrohistiocytic lesions(Kutzner, 1993). The tumor cells of fibrous histiocytomas (0/4) did not react with anti-CD34 antibody. All of the five dermatofibrosarcoma protuberans unequivocally demonstrated diffuse, strongly positive, and linear anti-CD34 staining of the tumor cells. Kutzner(Kutzner, 1993) also reported the same result in comparison between dermatofibrosarcoma protuberans and fibrous histiocytoma. Three cases of malignant fibrous histiocytoma were all negative.

CD34 positive dendritic cells were consistently identified within the endoneurium of normal nerve, all neurofibromas, and Antoni B (but not Antoni A) areas of neurilemmomas. CD34 was not expressed in all of the malignant peripheral nerve sheath tumors(Weiss and Nickoloff, 1993). It was suggested that CD34 defined a normally occurring nerve sheath cell that appears to be cytologically and immunophenotypically distinct from a fibroblast and conventional Schwann cell. These observations are consistent with the widely held view that neurofibromas consist of a varied population of cells ranging from conventional Schwann cells to fibroblasts, whereas the Antoni A areas of neurilemmomas are composed of a monomorphic population of Schwann cells(Weiss and Nickoloff, 1993). The antigen can also be localized to benign nerve sheath tumors, but tends to be lost in malignant ones. The consistent presence of CD34 within all 5 cases of dermatofibrosarcoma protuberans can be used as evidence in support of the view that these lesions are variants of nerve sheath tumors, and distinct from benign fibrous histiocytomas which consistently lack the antigen(Weiss and Nickoloff, 1993).

The biologic significance of CD34 positivity in epithelioid sarcoma is unclear. Watt et al. demonstrated previously that all CD34 antibodies are not equal and that differences in antibody reactivity can be attributed to the recognition of distinct epitopes by different CD34 reagents(Watt et al., 1987). It is not possible to tell with certainly whether the cells of smooth muscle and neural tumors and those of dermatofibrosarcoma protuberans actually contain CD34 antigen, or whether they are only cross reacting with the anti-CD34 antibody used in this study(Cohen et al., 1993).

In conclusion, antibody directed against the CD34 antigen can be used in formalin-fixed paraffin-embedded specimens and is not only being recognized for its usefulness in diagnosing vascular neoplasms, but also for the differential diagnosis of non-vascular mesenchymal lesions present in skin and soft tissue locations. As Weiss and Nickoloff(1993) stressed, further studies are indicated to determine if the histogenetic insights derived from patterns of CD34 immunoreactivity can be substantiated by additional morphological and functional analysis of these benign and malignant soft tissue neoplasms.

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