CD31 Immunoreactivity in Carcinomas and Mesotheliomas

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Abstract

CD31 is a specific and sensitive marker of endothelial differentiation. Previous reports have described its immunoreactivity in large series of soft tissue neoplasms, as well as its comparison with other available and commonly used endothelial markers. CD31 reactivity in carcinomas or mesotheliomas has been incompletely addressed, however. Hence, we applied anti-CD31 (JC70/A, DAKO, Carpinteria, Calif) to 290 previously characterized neoplasms by using a modified avidin-biotin-peroxidase complex technique following microwave epitope retrieval. Seven carcinomas showed plasmalemmal-based immunoreactivity (2 papillary thyroid carcinomas, 2 mucoepidermoid salivary gland carcinomas, 1 cutaneous adnexal tumor, 1 cutaneous squamous cell carcinoma, and 1 esophageal squamous cell carcinoma); the remaining 283 lesions were negative for this marker. We conclude that anti-CD31 immunostaining in carcinomas and mesotheliomas is rare. These findings support the concept that CD31 is a reliable marker of endothelial differentiation and should be included in diagnostic immunohistochemical panels when vascular tumors enter the differential diagnosis.

CD31 (platelet-endothelial cellular adhesion molecule, also known as PECAM-1), a 130-kd transmembrane glycoprotein and a member of the immunoglobulin "supergene" family, has been previously reported as a reliable marker of endothelial differentiation in formalin-fixed paraffin-embedded tissue sections.[1–5] The value of this reagent has substantially augmented the importance of immunohistochemistry to the diagnosis of vascular neoplasms, given the comparatively lower sensitivity and specificity of previously available vascular markers.[6–19]

The availability of a reliable endothelial marker assumes even greater importance in the context of aberrant keratin reactivity encountered in some endothelial and nonendothelial epithelioid soft tissue neoplasms, and the predilection of true epithelial tumors (especially their sarcomatoid variants) to exhibit immunoreactivity for vimentin.[17,20–24] The role of CD31 immunoreactivity has been incompletely addressed in the evaluation of carcinomas and mesotheliomas, tumors that clearly may enter the differential diagnosis of epithelioid and spindled angioproliferative lesions.[25,26] In one recent study of CD31 immunoreactivity,[5] weak cytoplasmic staining was seen in 2 of 16 mesotheliomas and 5 of 84 carcinomas; membrane staining was absent, however. The present study provides additional information about plasmalemmal-based CD31 immunoreactivity in a large series of carcinomas and mesotheliomas.

Materials and Methods

We obtained 290 carcinomas and pleural mesotheliomas from the archival files of the departments of pathology at Ohio State University Medical Center, Columbus, Ohio; the University of Virginia Health Sciences Center, Charlottesville; and, Washington University Medical Center, St Louis, Missouri. All cases were reviewed for diagnostic

accuracy by using accepted histopathologic and immunohistochemical criteria.

A representative formalin-fixed paraffin-embedded tissue block was identified for further immunohistochemical analysis. Five-micron sections were cut, heated to 55°C for 1 hour, deparaffinized in Americlear (American Scientific, McGaw Park, Ill), and dehydrated in graded alcohols. Endogenous peroxidase activity was quenched by immersion in absolute methanol containing 0.6% (vol/vol) hydrogen peroxide for 30 minutes. Slides were rehydrated in graded ethanols and distilled water. Epitope enhancement was achieved by immersing sections in a 0.01-mmol/L concentration of citrate buffer, pH 6.0, and heating in a commercial microwave oven for 8 minutes. After cooling to room temperature, sections were rinsed in phosphate-buffered saline, pH 7.4. The murine monoclonal anti-CD31 antibody JC70/A (DAKO, Carpinteria, Calif), diluted 1:20, was applied and incubated for 18 hours at 4°C in moisture chambers. Sections were developed by using a modified avidin-biotin-peroxidase complex technique[27] (VectaStain Elite, Vector Laboratories, Burlingame, Calif).

A chromogenic precipitate was obtained by immersion of slides in 3,3´-diaminobenzidine tetrahydrochloride (Sigma, St Louis, Mo), 0.5 mg/mL in phosphate buffer, pH 7.6, containing 0.0003% (vol/vol) hydrogen peroxide for up to 10 minutes. Slides were dipped in tap water, immersed briefly in osmium tetroxide (0.125% vol/vol), counterstained with Harris hematoxylin, and coverslipped using a synthetic mounting medium. Positive immunostaining was interpreted as membrane-based deposition of chromogen, with or without cytoplasmic accentuation. A formalin-fixed section of a capillary hemangioma served as a positive control. Negative controls were prepared by substitution of mouse ascites fluid for the primary antibody.

Results

The study group included 281 carcinomas and 9 pleural-based mesotheliomas **Table 1**. The carcinomas arose in a wide variety of primary sites. Of the 281 carcinomas, 7 exhibited membrane decoration with anti-CD31. Of these, 2 were papillary thyroid carcinomas, and 2 were low-grade mucoepidermoid salivary gland carcinomas. One example each of cutaneous squamous cell carcinoma, high-grade cutaneous adnexal carcinoma **Image 1**, and esophageal squamous cell carcinoma **Image 2**, also were positive for CD31. In each case, immunostaining was intense, although in the papillary thyroid neoplasms, reactivity was focal. Interestingly, the staining in the salivary gland tumors was confined to the epidermoid component and was similar to that seen in the examples of CD31$^+$ cutaneous squamous cell carcinoma and esophageal squamous cell carcinoma. Cytoplasmic accentuation of the membrane staining pattern was observed in the cutaneous lesions. The remaining 274 carcinomas and all 9 mesotheliomas failed to exhibit immunoreactivity of a membrane or cytoplasmic pattern. All negative and positive controls stained appropriately.

Discussion

CD31 has been touted as a sensitive and a specific marker of endothelial differentiation.[1–5] It has been studied in the context of soft tissue neoplasms in series of deeply situated and dermal-subcutaneous lesions.[3,4] The usefulness of this marker also has been the subject of an analysis of cutaneous angiosarcomas and carcinomas and mesotheliomas, comparing JC70/A (anti-CD31) with the antibodies My10/anti-CD34$^+$ and BNH9.[5] In the latter study, which included 100 carcinomas and mesotheliomas, weak cytoplasmic staining for CD31 was reported in isolated cases of carcinoma and mesothelioma; plasmalemmal-based immunoreactivity was reported to be absent.

Table 1
CD31 Immunoreactivity in Carcinomas and Mesotheliomas

Tumor Type	No. Positive/Total No.
Salivary gland	
Mucoepidermoid	2/5
Acinic cell	0/2
Adenoid cystic	0/4
Carcinoma ex pleomorphic adenoma	0/1
Skin	
Squamous cell	1/26
Basal cell	0/9
Adnexal	1/6
Thyroid	
Papillary	2/25
Follicular	0/11
Medullary	0/1
Pulmonary	
Adenocarcinoma	0/23
Squamous cell	0/5
Small cell undifferentiated	0/3
Naso/oropharyngeal and laryngeal squamous cell	0/9
Kidney	
Renal cell	0/9
Transitional cell	0/4
Hepatocellular	0/13
Gallbladder adenocarcinoma	0/6
Adrenal cortical carcinoma	0/2
Female reproductive tract	
Endometrial	0/8
Ovarian surface carcinoma	0/5
Cervical squamous cell	0/3
Pancreaticobiliary	0/19
Gastrointestinal	1/27
Prostate	0/16
Breast	0/27
Sarcomatoid (all sites)	0/12
Pleural mesothelioma	0/9

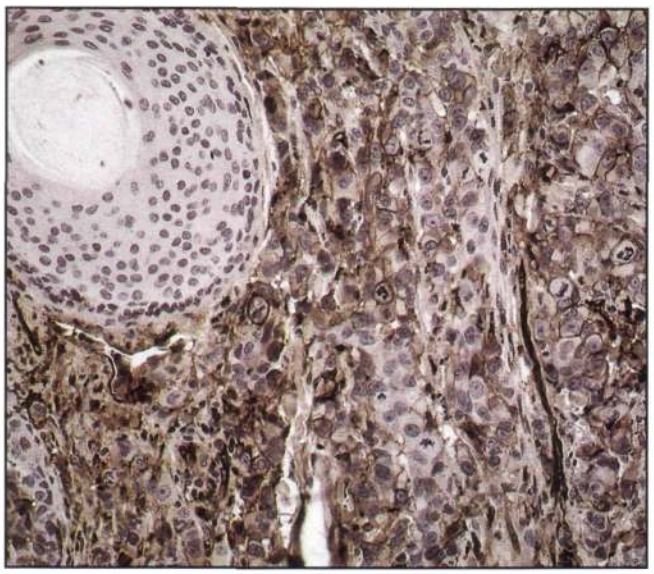

Image 1 Intense membrane-based staining with cytoplasmic accentuation in an example of poorly differentiated cutaneous adnexal carcinoma (anti-CD31 immunostain/hematoxylin counterstain, ×200).

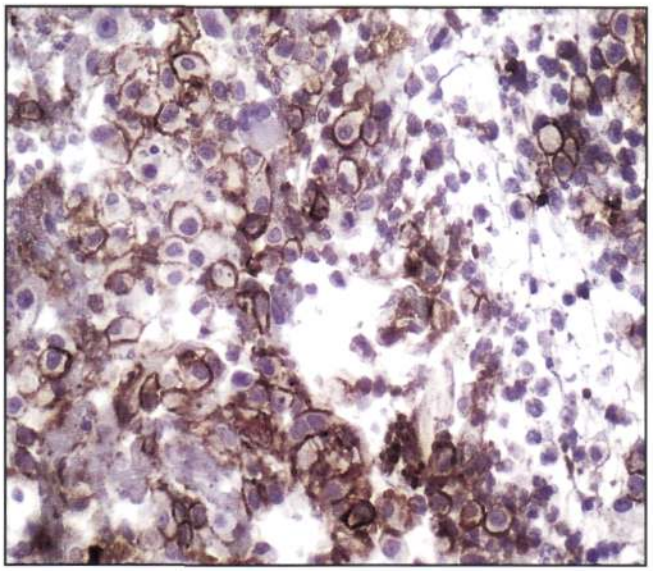

Image 2 Membrane-based immunoreactivity in the positive esophageal squamous cell carcinoma (anti-CD31 immunostain/hematoxylin counterstain, ×200).

The present study was designed to extend the analysis of CD31 immunoreactivity in a large series of carcinomas and mesotheliomas that can enter the differential diagnosis of vascular neoplasms.[24–26] The drastic differences in treatment and prognosis among vascular and nonvascular lesions reinforces the need for immunohistochemical markers that reliably separate endothelial neoplasms from their epithelial and mesothelial mimics.

The archival files of 3 institutions were used to construct a study group that covered the spectrum of carcinoma; in addition, 9 cases of mesothelioma were included. Our approach to CD31 immunoreactivity was identical to that previously published for deeply situated and cutaneous soft tissue neoplasms,[3,4] with the exception that microwave-based epitope retrieval was substituted for protease predigestion as the mode of antigen retrieval. Of 281 carcinomas, 274 were uniformly negative; all 9 mesotheliomas also failed to show immunoreactivity. Of the 7 carcinomas that were decorated by anti-CD31, 4 exhibited squamous differentiation. These included 1 cutaneous squamous cell carcinoma, 1 esophageal squamous cell carcinoma, and the squamous elements of 2 mucoepidermoid carcinomas arising in the parotid gland. The first of these is particularly relevant to the differential diagnosis of angiosarcoma in skin because of the tendency for some examples of acantholytic poorly differentiated cutaneous squamous cell carcinomas to assume a pseudoglandular or pseudovascular appearance.[25] Two of the remaining carcinomas exhibiting positive immunostaining were papillary thyroid carcinomas, while the third was a poorly differentiated adnexal carcinoma in the scalp. While the squamous neoplasms showed rather strong diffuse plasmalemmal-membrane–based staining, the immunostaining observed in the thyroid lesions was rather focal and seemed to be confined primarily to intercellular (lateral) instead of apical membranes. We saw no evidence of positive immunostaining in colonic adenocarcinoma or ductal carcinoma of the breast, which had been previously reported to exhibit some evidence of cytoplasmic anti-CD31 staining.[5]

The underlying reason for the apparent positivity obtained in rare sporadic cases of cutaneous, glandular, and visceral epithelial neoplasms is unknown. In the previous report of aberrant immunostaining using this marker, it was hypothesized that cross-reactivity with carcinoembryonic antigen (CEA) might account for the unexpected positivity.[5] This theory was based on biochemical similarities between CEA and CD31.[28] While it is conceivable that aberrant immunopositivity in the cases currently under study may be explained in this manner, the lack of a plasmalemmal membrane–based pattern of expression for CEA, together with the expected absence of this oncoprotein in papillary thyroidal neoplasms, mucoepidermoid carcinoma, and cutaneous squamous cell carcinoma argue against this as a complete explanation. Instead, it is possible that unexpected membrane-based immunopositivity for CD31 reflects abnormal expression of this nonepithelial adhesion moiety. This hypothesis of sarcomatoid or endothelial metaplasia previously has been postulated to explain the spectrum of epithelial and endothelial differentiation encountered in thyroid neoplasms.[29] Nevertheless, despite rare cases of CD31 reactivity in carcinoma, the results of the present study support and expand previous analyses that established the sensitivity and specificity of anti-CD31 antibodies

for endothelial differentiation in formalin-fixed paraffin-embedded human neoplasms. These results further reinforce the value of this marker for the immunohistochemical evaluation of epithelioid neoplasms when angiosarcoma is in the differential diagnosis.

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