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BRIEF METHOD

A New Monoclonal Antibody, D2-40, for Detection of Lymphatic Invasion in Primary Tumors

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Lymphatic invasion is a commonly reported histopathological finding in the assessment of primary tumors. In some studies, it has been found to be a predictor of shorter disease-free or overall survival in melanoma and cancers of breast, colon, and cervix (Birner et al, 2001; Borgstein et al, 1999; Clemente et al, 1992; Compton, 1999; Lauria et al, 1995). In the absence of effective immunohistochemical markers of lymphatic endothelium in paraffin sections, lymphatic invasion is currently identified on the basis of conventional hematoxylin and eosin (H&E) staining as the presence of tumor emboli within vascular channels distinctly lined by a single layer of endothelial cells. Pitfalls in the technique arise mainly from the difficulty in visualizing the lymphatic vessel wall following H&E staining. They include the inability to discern tumor emboli that obliterate the lumen of lymphatics and to distinguish retraction artifacts that isolate tumor aggregates due to tissue shrinkage during fixation from true tumor emboli in lymphovascular spaces. We presently report that monoclonal antibody (mAb) D2–40, a new selective marker of lymphatic endothelium (Kahn et al, 2002), clearly demarcates tumor emboli in lymphatics in paraffin sections of primary tumors, including cancers of breast, colon, prostate, cervix, endometrium, and skin (melanoma and squamous cell carcinoma). In a pilot study of 50 breast cancer cases, D2–40 identified lymphatic invasion in 44% lymph node-negative and 88% lymph node-positive cases. In comparison, we found that 18% of the specimens examined by H&E staining were false-negative and 4% were false-positive for detection of lymphatic invasion. This new mAb has the potential for increasing the accuracy of detection of lymphatic invasion in primary tumors and, therefore, could be widely applied for this purpose in surgical pathology.

MAb D2–40 (IgG1) to a Mr 40,000 0-linked sialoglycoprotein was purified from ascitic fluid, as previously described (Marks et al, 1999), and stored at a concentration of 0.7 mg/ml under sterile conditions at 4° C. Paraffin blocks of primary cancers of breast, colon, prostate, cervix, endometrium, and skin (melanoma and squamous cell carcinoma) were obtained from the Department of Pathology, Sunnybrook and Women's College Health Sciences Centre, University of Toronto. Sections of paraffin-embedded tissues (5 μm) were first incubated in methanol containing 3% H_2O_2 to inactivate endogenous peroxidase. For immunostaining, the sections were incubated with mAb D2–40 (0.1 ug/ml), followed sequentially by biotinylated goat anti-mouse immunoglobulin antibody (Zymed, San Francisco, California) at a 1:200 dilution and a horseradish peroxidase-avidin conjugate (Dako, Carpinteria, California) at a 1:500 dilution. For color development, the sections were incubated with 3:3 diaminobenzidine.

Examples of the application of D2–40 for the accurate detection of lymphatic invasion are shown in Figure 1. In Figure 1A, a tumor embolus that obliterates the lumen of a lymphatic in breast cancer is clearly outlined by positive immunostaining of the vessel wall with D2–40 (arrowhead). In Figure 1B, an adjacent H&E section shows the difficulty in discerning a tumor embolus. In Figure 1C, a retraction artifact that was initially spuriously diagnosed as lymphatic invasion on an H&E section in breast cancer was subsequently correctly identified as a retraction artifact on the basis of negative immunostaining with D2–40 (open arrowhead). Immunostaining was also negative for CD-31, CD-34 and factor VIII-related antigen (data not shown) ruling out a blood vessel tumor embolus. Neighboring lymphatics stain positively with D2–40 (arrows).

In Figure 1D, positive immunostaining with D2–40 outlines multiple lymphatics containing tumor emboli that obliterate the lumen (arrowheads) and uninvolved lymphatics containing lymphocytes (arrows) in colon cancer. In Figure 1E, positive immunostaining with

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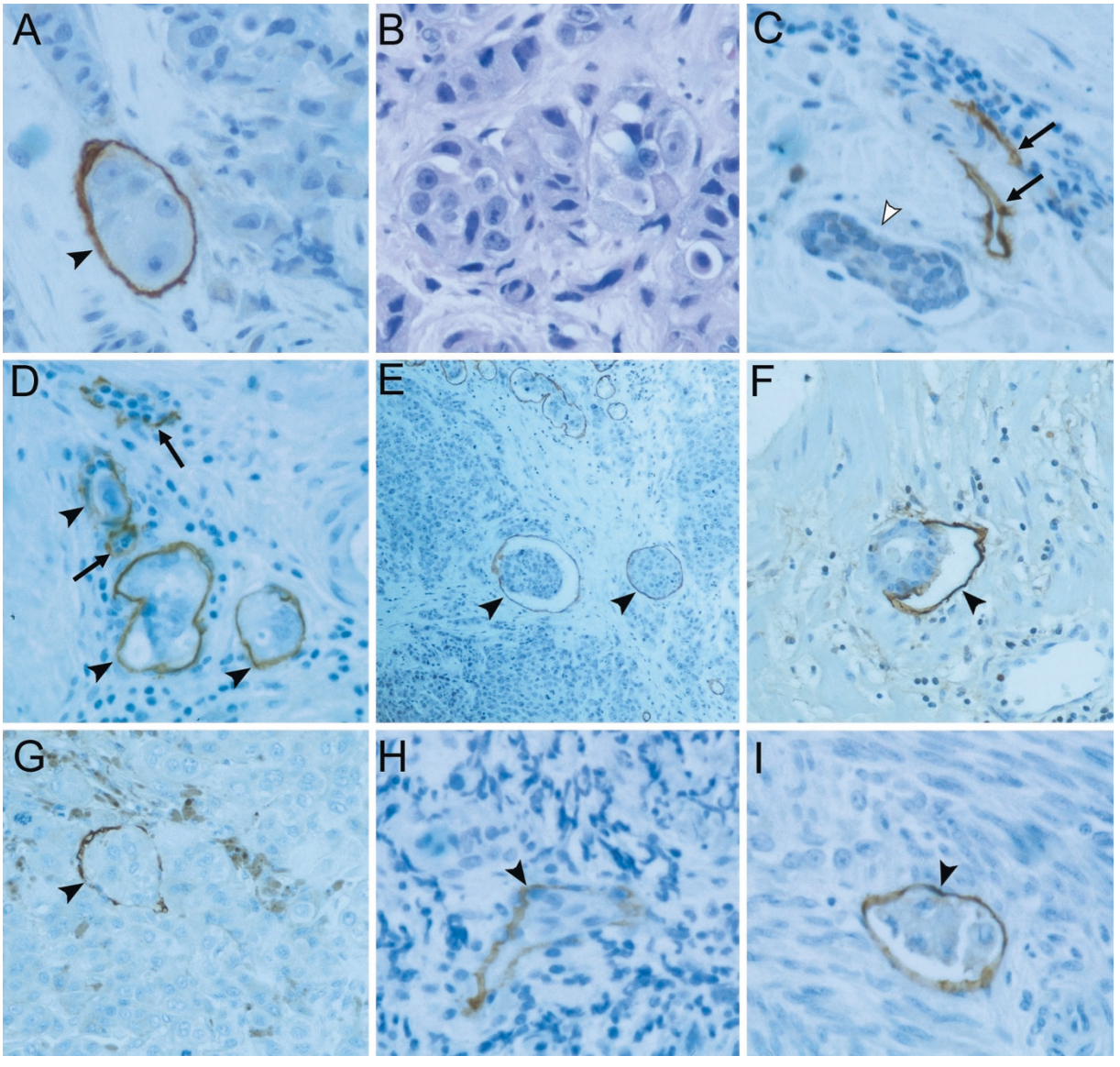

Figure 1.

Staining of primary tumor sections with D2–40 (A, C to I) or hematoxylin and eosin (B). Breast (A to C); colon (D); scalp (squamous cell carcinoma, E); prostate (F); skin (melanoma, G); cervix (H); endometrium (I). Positive immunostaining of lymphatic endothelium with D2–40 outlining tumor emboli in lumen of lymphatics (A, D, E, G to I) or invading vessel wall (F) is indicated by *arrowheads*. Absence of immunostaining with D2–40 around retraction artifact is indicated by *open arrowhead* in C. Positive immunostaining of lymphatic endothelium with D2–40 of uninvolved lymphatics is indicated by *arrows* in C and D. Note the presence of lymphocytes in the lumen of uninvolved lymphatics in D. Original magnification: A, B, D, F, G to I, ×300; C, ×200; E, ×100.

D2–40 outlines lymphatic tumor emboli (arrowheads) in squamous cell carcinoma of scalp; the one on the left is readily distinguished from a retraction artifact, and the one on the right is clearly identified even though it obliterates the lumen of the vessel. In Figure 1F, tumor cells invade the lumen of a lymphatic through the vessel wall staining positively with D2–40 (arrowhead) in prostate cancer. In Figure 1G, positive immunostaining with D2–40 identifies a tumor embolus (arrowhead) that completely obliterates the vessel lumen in melanoma. Brown melanin pigment present in some melanoma cells in the section was also seen on unstained slides (data not shown) and is not a product of the immunoperoxidase reaction. In Figure 1H and I, positive immunostaining with D2–40 outlines tumor emboli that completely or partially obliterate the vessel lumen in carcinoma of cervix (H) or endometrium (I), respectively. The comparison of detection of lymphatic invasion following conventional staining with H&E and immunostaining with D2–40 in 25 cases each of node-negative and node-positive breast cancer is shown in Table 1. Both the sensitivity and specificity of detection were higher for D2–40. Specimens examined by H&E were false-negative in 5/25 (20%) node-negative and 4/25 (16%) node-positive cases, respectively. Both groups had a false-positive rate of 1/25 (4%) by H&E.

The advent of selective immunohistochemical markers of lymphatic endothelium in paraffin sections will increase the accuracy of detection of lymphatic invasion in tumor specimens. Two recent communications describe the use of a polyclonal rabbit antibody to

Table 1. Comparison of Frequency of Lymphatic Invasion Detected by H&E and D2–40 in Invasive Ductal Carcinoma of Breast

	Lymph node negative (n = 25)		Lymph node positive (n = 25)	
Patient population	H&E	D2–40	H&E	D2–40
Lymphatic invasion (%)	6/25 (24)	11/25 (44)	18/25 (72)	22/25 (88)
Retraction artifact (%)	1/25 (4)		1/25 (4)	

podoplanin to identify lymphatic invasion in cancers of cervix and breast (Birner et al, 2001; Schoppmann et al, 2001). While both mAb D2–40 and the rabbit antibody to podoplanin are selective for lymphatic endothelium, the technical advantages of D2–40 over the latter reagent are as follows. First, D2–40 is monoclonal, while antipodoplanin is polyclonal. Second, D2–40 can be used directly, while antipodoplanin requires prior affinity purification using nitrocellulose strips containing recombinant protein. Third, D2–40 can be used to stain paraffin sections without the requirement for epitope retrieval, while antipodoplanin requires heat-dependent epitope retrieval for this purpose.

In summary, D2–40 is a new selective monoclonal immunohistochemical marker of lymphatic endothelium that can be used to detect lymphatic invasion in conventionally processed formalin-fixed and paraffin-embedded tissue specimens. The increased accuracy of detection of lymphatic invasion achieved by the use of this antibody will contribute to the evaluation of the utility of a qualitative and quantitative assessment of lymphatic invasion as a prognostic indicator in invasive cancer.

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References

Birner P, Obermair A, Schindl M, Kowalski H, Breitenecker G, and Oberhuber G (2001). Selective immunohistochemical staining of blood and lymphatic vessels reveals independent prognostic influence of blood and lymphatic vessel invasion in early-stage cervical cancer. Clin Cancer Res 7:93–97.

Borgstein PJ, Meijer S, and van Diest PJ (1999). Are locoregional cutaneous metastases in melanoma predictable? Ann Surg Oncol 6:315–321.

Clemente CG, Boracchi P, Andreola S, Del Vecchio M, Veronesi P, and Rilke FO (1992). Peritumoral lymphatic invasion in patients with node-negative mammary duct carcinoma. Cancer 69:1396–1403.

Compton CC (1999). Pathology report in colon cancer: What is prognostically important? Dig Dis 17:67–79.

Kahn HJ, Bailey D, and Marks A (2002). Monoclonal antibody D2-40, a new marker of lymphatic endothelium reacts with Kaposi's sarcoma and a subset of angiosarcomas. Mod Pathol 15:434–440.

Lauria R, Perrone F, Carlomagno C, De Laurentiis M, Morabito A, Gallo C, Varriale E, Pettinato G, Panico L, Petrella G, Bianco AR, and De Placido S (1995). The prognostic value of lymphatic and blood vessel invasion of operable breast cancer. Cancer 76:1772–1778.

Marks A, Sutherland DR, Bailey D, Iglesias J, Law J, Lei M, Yeger H, Banerjee D, and Baumal R (1999). Characterization and distribution of an oncofetal antigen (M2A antigen) expressed on testicular germ cell tumours. Br J Cancer 80:569–578.

Schoppmann SF, Birner P, Studer P, and Breiteneder-Geleff S (2001). Lymphatic microvessel density and lymphovascular invasion assessed by anti-podoplanin immunostaining in human breast cancer. Anticancer Res 21:2351–2356.