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Immunohistochemistry

Utility of immunostains for D2-40 in diagnostic pathology

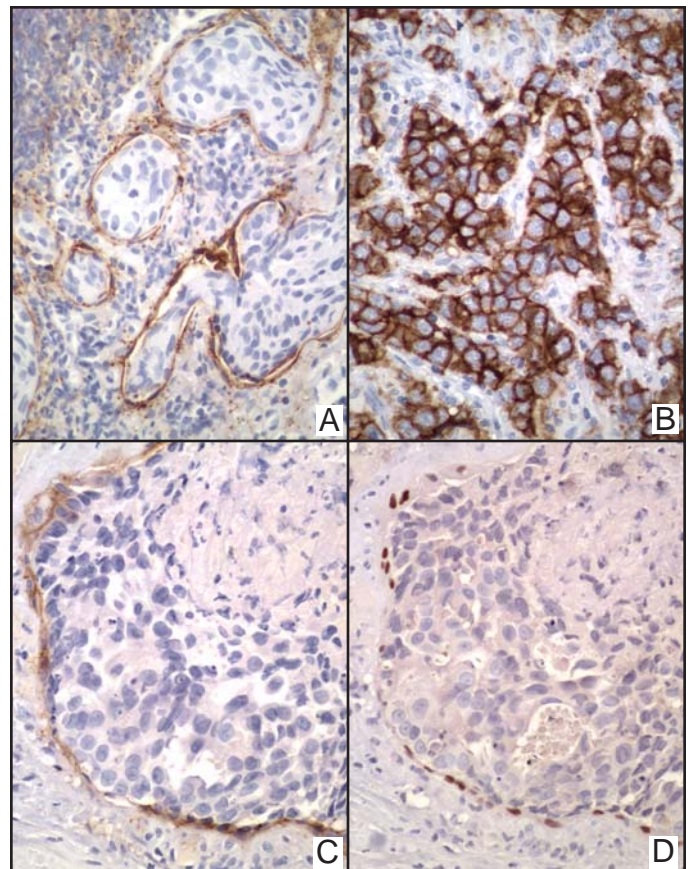
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by Rodney T. Miller, M.D., Director of Immunohistochemistry

Interest in D2-40 first centered on its use as a marker of lymphatic endothelium, since it does not stain vascular endothelium. As such, D2-40 immunostains have been used to improve the recognition of lymphatic invasion. In addition to staining of normal lymphatics throughout the body, Kaiserling reported reactivity with other normal cells, including follicular dendritic cells, interstitial cells of Cajal, a subset of dermal epithelial cells, myoepithelial cells (in breast and elsewhere), and prostate basal cells. Reactivity in tumors was also noted, including myofibroblastic tumors, GIST, mesothelioma, traumatic neuroma, seminoma, "other testicular tumors", pleomorphic adenoma, and follicular dendritic cell sarcoma.

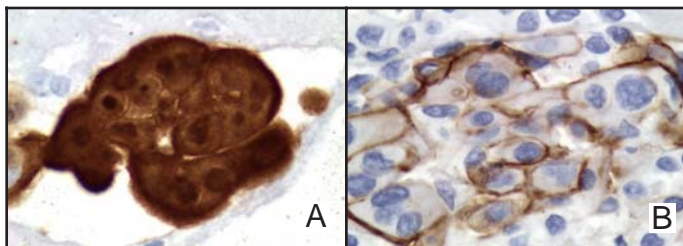
As might be expected, D2-40 stains tumors arising from lymphatics, as well as certain types of vascular tumors. Studies by Fukunaga and Kahn et al describe staining in lymphangioma (20/20), vascular transformation of lymph node sinuses (1/1), Dabska tumors (3/3), nearly all Kaposi's sarcomas (KS) (33/34), reactive angioendotheliomatosis (1/1), 10% of epithelioid hemangioendotheliomas (1/10), and a subset of angiosarcomas (10/22), particularly those with epithelioid or papillary morphology. Tumors lacking D2-40 included non-spindle cell hemangiomas (0/32), retiform hemangioendothelioma (0/1), Kaposiform hemangioendothelioma (0/1), angiolipomas (0/2), pyogenic granulomas (0/2), vascular malformations (0/2), hemangiopericytoma (0/1), and glomus tumors (0/8).

Although initial reports suggested that D2-40 immunostaining was specific for lymphatic endothelium and associated tumors arising from those cells, a study by Bellucci et al presented at the 2004 USCAP meeting (involving 20 cases of KS and 97 other spindle cell tumors) suggested otherwise. The authors identified 3 categories of tumors. Tumors that were frequently strong and diffusely (>40% of cells) positive included KS (100%, 20/20), schwannoma (100%, 9/9), meningioma



A: D2-40 stain highlighting lymphatic invasion in a squamous carcinoma of the tongue. B: Seminoma showing strong staining with D2-40. C: D2-40 staining of a breast biopsy, demonstrating "pseudo-lymphatic invasion", due to staining of myoepithelial cells by D2-40. D: Corresponding p63 stain on the breast biopsy, highlighting the myoepithelial cell nuclei.

(100%, 5/5), synovial sarcoma (100%, 7/7), GIST (83%, 5/6), and leiomyosarcoma (90%, 9/10). Another group was frequently but less intensely positive, and included solitary fibrous tumor (91%, 10/11), dermatofibroma (100%, 4/4), desmoid (100%, 5/5), spindle-cell melanoma (100%, 4/4), spindle-cell sarcoma (100%, 3/3), and malignant peripheral nerve sheath tumor (100%, 2/2). Dermatofibrosarcoma protuberans was infrequently and focally positive (31%, 7/18), as was



Calretinin (A) and D2-40 (B) on a mesothelioma. Note how the membrane pattern of D2-40 staining allows assessment of nuclear atypia, which is obscured on the calretinin stain.

neurofibroma (44%, 4/9).

Distinction of hemangioblastoma (HB) from metastatic renal cell carcinoma (RCC) involving the brain can be very challenging, particularly in patients with Von Hippel Lindau disease. Along those lines, Roy et al reported that D2-40 was negative in both primary and metastatic RCC of conventional clear cell type (0/28). In contrast, 83% (19/23) of HB showed strong membranous staining in vacuolated tumor cells, with 17% (4/23) showing weaker staining.

At the 2005 USCAP meeting, Liang et al presented data indicating that D2-40 may be useful in the distinction of primary adnexal carcinoma of the skin from adenocarcinoma metastasizing to the skin. Ninety-one cases were studied, including 77 primary cutaneous adnexal neoplasms of various types and 14 metastatic tumors to the skin, from carcinomas of the breast (6), lung (1), biliary tract (1), ovary (1), pancreas (1), colon (3), and thyroid (1). All of the primary skin adnexal carcinomas and most of the benign cutaneous adnexal tumors demonstrated variable D2-40 staining, but all of the metastases to the skin were negative. If study of additional cases confirms these preliminary findings, D2-40 may prove to be helpful in addressing this differential diagnostic problem.

D2-40 as a mesothelial marker has also been studied. In the study of Chu et al, membranous D2-40 reactivity was present in 96% (51/53) of mesotheliomas, and in 65% (17/26) of ovarian serous carcinomas, but was negative in pulmonary adenocarcinoma (n=30), RCC (n=35), breast carcinoma (n=16), prostate carcinoma (n=11), and urothelial carcinoma (n=7). Ordonez studied 40 mesotheliomas, 34 pulmonary carcinomas, and 81 nonpulmonary carcinomas. D2-40 was negative in 6 of 6

sarcomatoid mesotheliomas, but was present in 86% of epithelioid mesotheliomas (25/29), as well as in the epithelioid component of 80% of biphasic mesotheliomas (4/5). D2-40 was negative in all of the adenocarcinomas, but was reactive in the epithelioid component of 4 of 6 biphasic synovial sarcomas. Monophasic synovial sarcomas were negative.

D2-40 is now available at PROPATH. I have been pleased with its utility as a marker of lymphatics (provided you make certain you are not seeing cross reactivity with myoepithelial cells) and with its ability to stain mesotheliomas, particularly since its membranous pattern of staining does not obscure the cytologic features of the immunoreactive cells. This feature makes it easier to interpret the degree of cytologic atypia, which can be difficult in cells that are intensely stained by some of the other mesothelial markers, such as cytokeratin 5 and calretinin.

In summary, studies of D2-40 indicate that it is useful in recognizing lymphatic invasion, diagnosis of mesothelioma, and distinguishing hemangioblastoma from metastatic renal cell carcinoma. If further studies confirm initial observations, it may also assist in the distinction of cutaneous adnexal tumors from metastatic adenocarcinoma involving the skin, and may prove useful in the diagnosis of germ cell tumors.

References:

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Rodney T. Miller, M.D., Director of Immunohistochemistry

After completing an AP/CP residency and Surgical Pathology Fellowship, Dr. Miller spent 10 years in hospital-based Pathology, and set up and directed several hospital IHC labs. He joined PROPATH in 1993, and developed a large, sophisticated, and thriving IHC lab and IHC consultation service. He is a nationally and internationally recognized expert in the field, and has lectured on the subject numerous times. He has authored multiple scientific articles on IHC, and is a member of the Editorial Board of The American Journal of Clinical Pathology.

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The Leader in Pathology Services

8267 Elmbrook Dr, Ste 100
Dallas, Texas 75247-4009

(214) 638-2000

(800) 258-1253

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