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Original contribution

CD1a immunopositivity in perivascular epithelioid cell neoplasms: true expression or technical artifact? A streptavidin-biotin and polymer-based detection system immunohistochemical study of perivascular epithelioid cell neoplasms and their morphologic mimics

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Summary Perivascular epithelioid cell neoplasms comprise a family of rare neoplasms composed of morphologically distinctive perivascular epithelioid cells exhibiting a "myomelanocytic" immunophenotype. The distinction of perivascular epithelioid cell neoplasms from other tumors with melanocytic and smooth muscle differentiation can be difficult. A recent study has suggested that perivascular epithelioid cell neoplasms routinely express CD1a, a Langerhans cell–associated transmembrane glycoprotein involved in antigen presentation and that expression of this marker may be helpful in the distinction of perivascular epithelioid cell neoplasms from various mimics. We evaluated a series of perivascular epithelioid cell neoplasms and potential mimics for CD1a expression. A total of 54 cases (27 perivascular epithelioid cell neoplasms, 11 leiomyosarcomas, 10 melanomas, 6 clear cell sarcomas) were evaluated in 2 laboratories (Mayo Clinic Rochester: 31 cases, Carolinas Medical Center: 23 cases). Selected positive cases were retested at Carolinas Medical Center (11 cases) and Mayo Clinic Rochester (10 cases). Mayo Clinic Rochester methods were as follows: MTB1 clone (1:20, Novocastra, Newcastle-upon-Tyne, UK), heat-induced epitope retrieval in EDTA (pH 8.0), and Dako Advance detection system (Dako Corp, Carpinteria, CA) with background-reducing diluent. Carolinas Medical Center methods were as follows: MTB1 clone (1:30; CellMarque, Rocklin, CA), heat-induced epitope retrieval in Medium Cell Conditioner #1 (pH 8.0-9.0), and streptavidin-biotin detection system with diaminobenzidine chromogen, with and without biotin blocking. Scores were as follows: 1+, 5% to 25%; 2+, 26% to 50%; and 3+, more than 51%. Langerhans cells served as a positive internal control in all tested cases. All Mayo Clinic Rochester cases were negative. Sixteen Carolinas Medical Center perivascular epithelioid cell neoplasms (14 renal angiomyolipomas, 1 soft tissue perivascular epithelioid cell neoplasm, 1 pulmonary clear cell "sugar" tumor) showed CD1a immunopositivity (1+: 7 cases; 2+: 7 cases; 3+: 2 cases) when tested without biotin blocking, 11 of these cases were retested with biotin blocking and were negative. All non–perivascular epithelioid cell neoplasms were negative. All positive perivascular epithelioid cell neoplasms showed cytoplasmic staining only, without membranous staining. Ten Carolinas Medical Center positive perivascular epithelioid cell neoplasms were negative when retested a Mayo Clinic Rochester, using a polymer-based detection system. We conclude that perivascular epithelioid cell neoplasms do not truly express CD1a in

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a biologically plausible membranous pattern, but may instead show aberrant cytoplasmic immunopositivity in some laboratories. Close inspection of published photomicrographs of previously reported CD1a-positive perivascular epithelioid cell neoplasms shows an identical pattern of cytoplasmic positivity, likely reflecting abundant endogenous biotin within perivascular epithelioid cell neoplasm cells. We do not believe that there is a role for CD1a immunohistochemistry in the differential diagnosis of perivascular epithelioid cell neoplasms.

1. Introduction

Perivascular epithelioid cell neoplasms (PEComas) comprise a family of morphologically distinctive tumors showing a unique "myomelanocytic" immunophenotype, with coexpression of muscle markers, such as smooth muscle actins, and melanocytic markers, such as gp100 protein (identified by monoclonal antibody [mAb] HMB45) and Melan-A [1]. The PEComa family of tumors includes angiomyolipoma (AML), clear cell "sugar" tumor of the lung (CCST), lymphangioleiomyomatosis (LAM), and a number of unusual visceral, intra-abdominal and soft tissue/bone tumors, described under a variety of different names. There is a strong association between AML and LAM and the tuberous sclerosis complex, although this link is less strong for other members of the PEComa family [2].

Although the diagnosis of renal AML and pulmonary LAM is typically straightforward, the diagnosis of other PEComas may be considerably more challenging, owing to the considerable overlap between their morphologic and immunohistochemical features with those of true smooth muscle tumors and tumors showing melanocytic differentiation, such as melanoma and clear cell sarcoma (CCS). There has thus been continued interest in the development and application of novel immunohistochemical markers to assist in the differential diagnosis of PEComas.

Recently, Adachi and colleagues [3, 4] have suggested that expression of CD1a, a Langerhans cell–associated cell surface glycoprotein, may be useful in the diagnosis of PEComas, noting its expression first in a single case of CCST and then in a larger follow-up study of 19 PEComas, including renal/hepatic AML, uterine LAM, and pulmonary CCST. Most recently, Fadare and Liang [5] have shown a series of uterine epithelioid smooth muscle tumors of conventional type to be CD1a negative, suggesting a possible role for CD1a immunohistochemistry in this sometimes difficult differential diagnosis. Prompted by these findings, we examined CD1a expression in a series of well-characterized PEComas and potential morphologic mimics.

2. Methods

Cases were collected from the surgical pathology archives of Mayo Clinic, Rochester, MN (MCR) and the Carolinas Medical Center Charlotte, NC (CMC), and from the consultation archives of one of the authors (A.L.F.). Twenty-seven PEComas were identified, including 18 renal AML, 8 PEComas arising in soft tissue/bone locations, and a single pulmonary CCST. All cases of PEComa showed appropriate morphology and had been previously shown to coexpress smooth muscle actins and melanocytic markers (eg, HMB45,

Table 1 Immunohistochemical methods and sources

	MCR	CMC	Adachi et al [4]
Tissue type	FFPE	FFPE	FFPE
CD1a antibody	Clone MTB1 (Novocastra, Newcastle-upon-Tyne, UK)	Clone MTB1 (CellMarque, Rocklin, CA)	Clone O10 (MBL, Marseille, France)
Dilution	1:20	1:30	Prediluted
Epitope retrieval	30 min in 97C EDTA, pH 8.0 in Dako Corp PT Link Module (Dako Corp)	Medium Cell Conditioner #1 (pH 8.0-9.0; Ventana Medical Systems, Tucson, AZ) for 8 min at 95°C and 20 min at 100°C	"Performed according to the manufacturer's instructions."
Detection system	Dako ADVANCE with Dako ADVANCE HRP and DAB (Dako Corp)	Ventana amplifiers A and B, biotin immunoglobulin, streptavidin HRP, and DAB	Streptavidin-peroxidase complex kit (Dako, Glostrup, Denmark) and DAB
Endogenous peroxidase blocking	3% hydrogen peroxide/absolute methanol for 10 min	Not performed/performed [a]	Not reported
Endogenous biotin blocking	Nonbiotin detection system	Not performed/performed [a]	Not reported

Abbreviations: FFPE, formalin-fixed, paraffin-embedded tissue blocks cut in 4 μmol/L sections; HRP, horseradish peroxidase; DAB, diaminobenzidine.
[a] A subset of cases evaluated at CMC were subjected to an additional biotin blocking step, as detailed in the "Methods" section.