Renal Angiomyolipoma

Further Immunophenotypic Characterization of an Expanding Morphologic Spectrum

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● Background.—Renal angiomyolipoma is a benign tumor histologically characterized by proliferation of spindle cells, epithelioid cells, and adipocytic cells in concert with many thick-walled blood vessels. To add further diagnostic confusion, an epithelioid cell–predominant variant of renal angiomyolipoma has recently been described. HMB-45 immunoreactivity correlates with ultrastructural striated organelles that closely resemble premelanosomes, although no evidence of melanogenesis has been documented in this tumor.

Objective.—To further characterize the immunophenotypic and ultrastructural profile of renal angiomyolipoma based on phenotypic cell type (epithelioid, spindle, and adipocytic cell).

Design.—Formalin-fixed, paraffin-embedded tissues from 27 renal angiomyolipomas and 8 renal cell carcinomas were immunostained with monoclonal antibodies to the melanoma-associated antigens HMB-45, HMB-50, NKI/C3 (CD63), and tyrosinase; the smooth muscle–related antigens calponin and muscle-specific actin (HHF-35); S100; and cytokeratin (CK). All renal angiomyolipomas were also immunostained with a polyclonal antibody to renin. Ultrastructural examination was performed on 9 selected cases.

Results.—All renal angiomyolipomas stained positive for HMB-45, HMB-50, NKI/C3, muscle-specific actin (HHF-35), and calponin. Overall, HMB-45, HMB-50, and NKI/C3 preferentially stained the epithelioid cells. Tyrosinase staining was present in 50% of the renal angiomyolipomas with adequate tissue for staining (12 of 24 cases); positive staining and intensity paralleled HMB-45, HMB-50, and NKI/C3. Muscle-specific actin (HHF-35) and calponin preferentially stained the spindle cells. The adipocytic cells stained positive for both melanoma-associated antigens and smooth muscle antigens. Epithelioid cells, spindle cells, and adipocytic cells were CK, S100, and renin negative. Ultrastructural findings paralleled immunohistochemical staining patterns. Premelanosome-like organelles and electron dense granules were more readily detected in the epithelioid cells within the tumor, whereas ultrastructural characteristics of smooth muscle cells were more easily found in the spindle cells. All renal cell carcinomas stained positive for CK, NKI/C3 staining was variable, and all were negative for HMB-45, HMB-50, smooth muscle actin (HHF-35), and calponin.

Conclusion.—In renal angiomyolipoma, the epithelioid and spindle cells have preferential staining patterns for melanoma-associated antigens versus smooth muscle antigens, respectively. Positivity in renal angiomyolipoma for HMB-50, NKI/C3, and tyrosinase, in addition to HMB-45, provides evidence for the presence of different melanoma-associated gene products. Immunophenotypic overlap of the 3 histologically distinct renal angiomyolipoma cell populations suggests a common cell line, supporting a unitarian concept for renal angiomyolipoma. Ultrastructural characteristics of the 3 renal angiomyolipoma cell phenotypes parallel the immunophenotype, giving further support to a common cell line. Our study lends further credence to the perivascular epithelioid cell concept as proposed by Bonetti and colleagues.

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Renal angiomyolipoma is an unusual, generally benign tumor that has been associated with tuberous sclerosis and is characterized by a histologic triad of tortuous, thick-walled blood vessels, phenotypic smooth muscle cells, and adipose cells in varying proportions. Recent studies have presented an interesting immunophenotypic and ultrastructural profile of renal angiomyolipoma. Immunoreactivity for HMB-45 antigen, a melanosome-associated protein, has been demonstrated in renal angiomyolipoma, as has immunoreactivity for the muscle-specific actin antigen HHF-35.1–11 By transmission electron microscopy, premelanosome-like crystalloid structures have been

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identified in the cytoplasm of the smooth muscle cell component of renal angiomyolipoma.7 In addition, cytoplasmic periodic acid–Schiff–positive diastase-resistant rhomboid crystals, which are negative for renin immunoreactivity, have been identified in renal angiomyolipoma.12 Concurrent investigations have demonstrated HMB-45 immunoreactivity in a group of other unusual tumors, including renal leiomyomas, hepatic angiomyolipoma, uterine angiomyolipoma, pulmonary lymphangioleiomyomatosis, clear cell (sugar) tumor of the lung, and clear cell (sugar) tumor of the pancreas.3,6,13±23

These recent immunophenotypic and ultrastructural findings, as well as recent case reports of an epithelioid cell–predominant variant of renal angiomyolipoma (Figure 1), have led to an increased interest in this unusual tumor.3,9,24 The purpose of this study was to further characterize renal angiomyolipoma based on immunohistochemical and ultrastructural examination of the 3 cell types comprising renal angiomyolipoma, namely, the epithelioid cells, the phenotypic smooth muscle spindle-type cells, and the adipocytic cell population.

MATERIALS AND METHODS

Twenty-seven renal angiomyolipomas were retrieved from the surgical pathology files of the Henry Ford Hospital and the University of Texas M. D. Anderson Cancer Center; all cases were histologically confirmed, and representative formalin-fixed, paraffin-embedded tissue was selected from each case for immunohistochemical evaluation. Because of the reported pitfall of mistaking the epithelioid cell–predominant variant of renal angiomyolipoma for renal cell carcinoma, 8 renal cell carcinoma control cases, with epithelioid cells having abundant eosinophilic cytoplasm representing (solid) chromophil, chromophobe, clear cell, or not otherwise specified types, were also included. Immunoreactivity to melanoma-associated antigens was examined using monoclonal antibodies to HMB-45, HMB-50, NKI/C3, and tyrosinase. Immunoreactivity to muscle-associated antigens was examined using monoclonal antibodies to muscle-specific actin (HHF-35) and calponin. Immunoreactivity to S100 protein, a feature associated with normal adipocytes and benign lipomatous tumors and melanocytic, schwannian, and myoepithelial cells, was examined using a monoclonal antibody. Immunoreactivity to cytokeratin (CK) was examined using a pool of 5 monoclonal antibodies directed against both high- and low-molecular-weight CKs. As an adjunct test, immunoreactivity to renin was examined in the renal angiomyolipoma cases using a renin-specific polyclonal antibody.

Formalin-fixed, paraffin-embedded tissue sections, 4 to 5 μm thick, were deparaffinized and rehydrated in graded concentrations of ethanol to distilled water. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide followed by a brief rinse in distilled water and a wash in phosphate-buffered saline. Tissue sections underwent pepsin enzyme digestion before an immunohistochemical evaluation. Some sections were then lightly counterstained with Mayer hematoxylin. Tissue sections stained for calponin (Table) underwent enzyme digestion with 0.01% Pronase (Calbiochem, San Diego, Calif) before microwave oven heat–induced epitope retrieval.25 Sections were then incubated with primary antibody anticalponin for 45 minutes at room temperature. Antibody localization was performed using avidin-biotin complex immunoperoxidase method, using the Strept AB complex (Dako Corporation, Carpinteria, Calif), diluted 1:100. Enzymatic staining was performed using the chromogen 3 amino-9-ethylcarbazol. Sections were then lightly counterstained with Mayer hematoxylin.

Tissue sections stained for calponin (Table) underwent enzyme digestion with 0.01% Pronase (Calbiochem, San Diego, Calif) before microwave oven heat–induced epitope retrieval.25 Sections were then incubated with primary antibody anticalponin for 45 minutes at room temperature. Antibody localization was performed using avidin-biotin complex immunoperoxidase method, using the Strept AB complex (Dako Corporation, Carpinteria, Calif), diluted 1:100. Enzymatic staining was performed using the chromogen 3 amino-9-ethylcarbazol. Sections were then lightly counterstained with Mayer hematoxylin.

Tissue sections stained for renin (Table) were submitted to a polyclonal antibody directed against human renin, using a peroxidase antiperoxidase method. The sections were then incubated with the antirenin serum (1:1000) for 60 minutes, followed by incubations with a swine anti-rabbit serum and the rabbit peroxidase antiperoxidase complex as previously described.26–27 DAB was used as chromogen. Sections were then counterstained with methyl green.

Immunoreactivity for HMB-45, HMB-50, NKI/C3, tyrosinase, muscle-specific actin (HHF-35), calponin, CK, S100, and renin was determined qualitatively. Immunopositive tumor cells demonstrated definite, granular cytoplasmic staining. In addition, the tumors were evaluated for cell type staining preference (epithei-
RESULTS

All renal angiomyolipomas stained positively for the melanoma-associated antigens HMB-45, HMB-50, and NKI/C3. Immunoreactivity for HMB-45 and HMB-50 was semiquantitatively similar. Immunoreactivity for HMB-45, HMB-50, and NKI/C3 was detected in the epithelioid cell, spindle-type cell, and adipocytic cell components of renal angiomyolipoma (Figure 2, a and b). HMB-45, HMB-50, and NKI/C3 antibodies preferentially stained the epithelioid cells; this was most pronounced in cases of epithelioid cell–predominant renal angiomyolipoma. Immunoreactivity to NKI/C3 tended to be more pronounced compared with HMB-45 and HMB-50. HMB-45– and HMB-50–positive staining was limited to renal angiomyolipoma, whereas positive staining for NKI/C3 was present in some of the cases of renal cell carcinoma. This positive staining for NKI/C3 in renal cell carcinoma was qualitatively much less intense than that detected in renal angiomyolipoma. Normal kidney and perirenal tissues were negative for HMB-45, HMB-50, and NKI/C3 staining. Twelve of 24 cases of renal angiomyolipoma stained positively for tyrosinase. Tyrosinase preferentially stained the epithelioid cells; tyrosinase staining intensity was qualitatively less than HMB-45, HMB-50, or NKI/C3 but paralleled the staining pattern for these antigens (Figure 3).

All renal angiomyolipomas stained positively for the smooth muscle–associated antigens muscle-specific actin (HHF-35) and calponin. Staining intensity for both smooth muscle–associated antigens muscle-specific actin (HHF-35) and calponin was both more intense and more extensive (Figure 4, a and b). Immunoreactivity for muscle-specific actin (HHF-35) was both more intense and more extensive in renal angiomyolipoma compared with calponin. Positive staining for muscle-specific actin (HHF-35) and calponin was limited to renal angiomyolipoma and normal vascular structures. Renal cell carcinoma tumor cells and normal kidney epithelial cells stained negative for muscle-specific actin (HHF-35) and calponin.

All renal angiomyolipomas were negative for S100 staining; in particular, the adipose-type cells did not stain for S100. All renal angiomyolipomas were negative for renin immunoreactivity. CK immunostaining detected entrapped renal tubules at the periphery of many of the renal angiomyolipomas, adjacent to the tumor-renal parenchymal interface. The renal angiomyolipoma tumor cells and the associated blood vessels were negative for CK immunoreactivity.

All renal cell carcinomas were immunoreactive to CK and stained negative for the melanoma-associated antigens HMB-45 and HMB-50, the smooth muscle–associated antigens muscle-specific actin (HHF-35) and calponin, and renin. NKI/C3 immunoreactivity in renal cell carcinoma was variable, with 5 (63%) of 8 renal cell carcinomas having at least moderate positive staining for NKI/C3 (38% moderate staining, 25% extensive staining). The adjacent, normal renal parenchyma present in all cases of both renal angiomyolipoma and renal cell carcinoma stained negative for HMB-45, HMB-50, NKI/C3, and renin antigens; adjacent renal epithelium and blood vessels appropriately stained positive for CK and the smooth muscle antigens muscle-specific actin (HHF-35) and calponin, respectively, and served as built in positive controls.

Nine cases of renal angiomyolipoma were examined ultrastructurally. Foci with spindle cell predominance (3 cases), foci with epithelioid cell predominance (3 cases), and foci with adipocytic cell predominance (3 cases) were selected for ultrastructural study. The distribution of spindle cells was closely associated with vascular wall smooth muscle cells, whereas adipocytic cells imperceptibly merged with the epithelioid cells. Electron microscopic examination of spindle cells, in renal angiomyolipoma, revealed ultrastructural characteristics of smooth muscle cells. These cells were enveloped by external lamina that was fused and interanastamosing in areas populated predominantly by epithelioid cells. The spindle cells, compared with the epithelioid cells, contained more microfilaments with occasional dense bodies, subplasmalemmal dense plaques, and pinocytic vesicles, characteristic of smooth muscle cells (Figure 5). There were also cells transitional between spindle cells and epithelioid cells, with relatively fewer electron dense granules, swollen mitochondria, and occasional stacks of rough endoplasmic reticulum. These ultrastructural features suggest modified smooth muscle cells. Epithelioid cells were closely apposed with interdigitating cytoplasmic processes, occasional desmosome-like junctional complexes, and uniform, narrow intercellular spaces. The epithelioid cells contained abundant rough endoplasmic reticulum, occasional actinlike thin filaments, and microtubules. Some of the epithelioid cells contained concentric membranous endoplasmic reticulum, numerous mitochondria, and cytoplasmic glycogen granules (Figure 6, a). Numerous polymorphic electron dense lysosomes and occasional prelamellar–like crystallloid structures were present in the epithelioid cells (Figure 6, b). In the adipocytic cells, the cytoplasm was occupied by homogenous osmophilic lipid vacuoles that were not membrane bound; occasional cells were multivacuolar (Figure 7). Rare electron dense granules and microfilaments were seen in these adipocytic cells.

The 27 patients (20 women and 7 men) with renal angiomyolipoma ranged in age from 29 to 75 years, with an average age of 56.6 years (SD, 15.7 years). Of the 27 renal angiomyolipomas, 10 arose on the right side and 17 arose on the left side; average tumor size was 4.8 cm (SD, 3.1 cm; range, 0.8–12 cm). No cases had metachronous or synchronous bilateral renal angiomyolipomas, and no cases had more than one focus of renal angiomyolipoma per kidney. A single patient, a 29-year-old woman with a 9.5-cm, left-sided angiomyolipoma, had clinical evidence of tuberous sclerosis. A second patient, a 32-year-old man with a 3-cm, left-sided renal angiomyolipoma, had cerebral palsy; all other patients had unremarkable neuromuscular and dermatologic findings.
COMMENT

Renal angiomyolipoma is an unusual tumor of uncertain histogenesis. Many authors have considered it to be a hamartomatous lesion. Because of a diversified phenotypic population, a cell of origin of this tumor has been the subject of many studies. Recent detection of clonal genomic alterations in renal angiomyolipoma and rare case reports of malignant renal angiomyolipomas are features that favor a neoplasm.28-33 The incidence of renal angiomyolipoma, based on a recent employee screening ultrasound study of 17,941 healthy adults, appears to be approximately 0.22% for women and 0.1% for men; none of these ultrasonographically detected renal angiomyolipomas were associated with tuberous sclerosis.34 An earlier, autopsy-based study of 8501 patients reported an overall renal angiomyolipoma incidence of 0.32%.35 Our study demonstrates a similar female predominance for renal angiomyolipoma (20 women and 7 men) and a similar relative lack of patients with tuberous sclerosis in the study population.

Perhaps the principal cell population of renal angiomyolipoma is the spindle cells intimately associated with the vascular walls. These spindle cells, in renal angiomyolipoma, are immunoreactive for calponin, as demonstrated in this study. Calponin is a 34-kd protein that interacts with F-actin and tropomyosin in a calcium-independent manner and with calmodulin in a calcium-dependent manner.36,37 Calponin appears to be restricted to smooth muscle cells in adult tissues; embryologic studies have detected calponin messenger RNA in cardiac tube development and endothelial cells. Calponin messenger RNA has also been detected in myoepithelial cells in the prepubertal stage of breast development.38-41 Renal angiomyolipoma is also immunoreactive for muscle-specific actin (HHF-35) antigen. Actin is a major structural protein that plays a role in cell structure and has a contractile function. Muscle-specific actin is found in, but not restricted to, smooth

Figure 5. Renal angiomyolipoma: spindle cell with smooth muscle cell ultrastructural characteristics, including microfilament with dense plaque (arrowhead) (original magnification ×2500).

Figure 6. a. Renal angiomyolipoma: epithelioid cells with concentric membranous body of endoplasmic reticulum and cytoplasmic glycogen particles are surrounded by external lamina. Note subplasmalemmal dense plaques in these epithelioid cells (arrowhead) (original magnification ×8000). b. Renal angiomyolipoma; premelanosome-like organelles (arrowhead) in epithelioid cell with junctional complex (arrow) (original magnification ×25,000).

Figure 7. Renal angiomyolipoma; univacuolar and multivacuolar cells with cytoplasmic filaments (arrow) (original magnification ×3150).
muscle cells; muscle-specific actin can also be detected, immunohistochemically, in myoepithelial cells, myofibroblasts, skeletal muscle, and various tumors with either myoepithelial, myofibroblastic, or muscle differentiation.42,43

Demonstration of both calponin and muscle-specific actin (HHF-35) immunoreactivity in renal angiomyolipoma further characterizes the smooth muscle cell nature of this unusual tumor; this is supported by ultrastructural study. The spindle-type cells in renal angiomyolipoma preferentially stained positive for the muscle markers calponin and muscle-specific actin (HHF-35). This preferential staining pattern paralleled the ultrastructural characteristics of the 3 cell types in that, quantitatively, the spindle-type cells contained more numerous microfilaments than either the epithelioid cells or the adipocytic cells. The lack of S100 staining makes it unlikely that these cells are myoepithelial in origin. The lack of either Z-bands or Weibel-Palade bodies, ultrastructurally, makes it unlikely that these cells are of either skeletal muscle or endothelial cell origin.

This study, based on 27 cases of renal angiomyolipoma, supports the findings of previous studies that demonstrated HHF-35 and HMB-45 immunoreactivity in renal angiomyolipoma and that described the presence of premelanosomes by ultrastructural examination.1,3,5–12 In addition, this study expands and further characterizes, overall and based on cell type, the melanosome and melanoma and smooth muscle immunophenotype and ultrastructural features of renal angiomyolipoma. The predominant cell population in most renal angiomyolipomas is the spindle cells recognized immunohistochemically and ultrastructurally as smooth muscle cells. An additional but interesting cell population in renal angiomyolipoma is epithelioid cells, which can be a predominant cell population in some cases. The cell of origin and the striking immunoreactivity of the epithelioid cells to melanoma-associated antigen remain an enigma. The epithelioid cells have higher sensitivity for melanocytic tumors than HMB-45 but is less specific and has been noted to be immunoreactive in some epithelial tumors, including neuroendocrine tumors and rare breast, ovarian, and lung carcinomas.49 This study demonstrated extensive NKI/C3 immunoreactivity in most renal angiomyolipomas; however, weak positive staining for NKI/C3 was noted in most renal cell carcinomas, thereby limiting the utility of NKI/C3 in immunohistochemically differentiating renal angiomyolipoma from renal cell carcinoma. Of interest, it has been recently reported that angiomyolipomas stain positive for A103, an antibody to Melan-A (Mart-1).50 Melan-A immunoreactivity has been detected in melanocytes; however, Melan-A has also been detected in adrenocortical tumors and in other steroid tumors, including Sertoli-Leydig cell tumors and Leydig cell tumors.51 Fifty percent of the renal angiomyolipomas stained at least focally positive for tyrosinase. Tyrosinase facilitates the oxidation of phenolic substrates in the synthesis of melanin and, in vertebrates, is limited to specialized cells.52–55 Positive immunostaining for tyrosinase was less intense than HMB-45, HMB-50, and NKI/C3 but paralleled these other markers and preferentially stained the phenotypic epithelioid cells. The positive staining of the adipocytic cells for both the melanosome and melanoma markers and the smooth muscle markers coupled with negative staining for S100 suggest that the adipocytic cells in renal angiomyolipoma may represent fatty metamorphosis within a single cell type. Although rare electron dense granules were identified ultrastructurally in the adipocytic cells, the relative lack of ultrastructural findings in the adipocytic cells may reflect a sampling phenomenon (“needle in a haystack”) whereby the fat represents most of the cytoplasmic volume, effectively dispersing cytoplasmic organelles over a larger cell volume. This interpretation is supported by the positive immunostaining for both melanocytic and smooth muscle markers in these fat-containing cells.

Bonetti and colleagues13,44 in 1992 and 1994, suggested that renal angiomyolipoma and clear cell tumor of the lung had in common an unusual epithelioid cell type characterized by clear and acidophilic cytoplasm, immunoreactivity with melanocytic markers, and a perivascular distribution; they proposed the term perivascular epithelioid cell (PEC) to identify this cell. In 1996, Zamboni and colleagues,23 including Bonetti, proposed a modulation schema encompassing the morphologic-immunophenotypic variability in tumors composed of PEC, including angiomylipomas, lymphangioleiomyomas, and sugar tumors of the lung and pancreas. Bonetti46 further clarified this unifying concept of PEC in 1997. Our results support this modulation schema of PEC in renal angiomyolipoma. The
shared immunoreactivity and ultrastructural characteristics of the 3 cell types (epithelioid, spindle, adipose) in renal angiomyolipoma suggest a single cell lineage. The morphologic differences and preferential staining patterns of the 3 cell types suggest secondary differentiation (Figure 8). The ultrastructural findings, in concert with the immunohistochemical profile of renal angiomyolipoma, suggest that renal angiomyolipoma is a tumor composed of modified smooth muscle cells with secondary morphoimmunophenotypic differentiation. Recently, Tsui and colleagues have demonstrated morphologic patterns and immunohistochemical staining in a study of 30 hepatic angiomyolipomas that parallel our findings in renal angiomyolipomas. Current ultrastructural and immunophenotypic studies of other unusual, HMB-45 immunoreactive-associated lesions, such as clear cell tumor of the lung, suggest that these tumors may also be composed of a similar modified smooth muscle cell.

Immunohistochemical detection of melanoma- or melanosoma-associated proteins in smooth muscle or modified smooth muscle is still enigmatic, yet there is no documented record for actual melanin formation in these cells. Pigmented myomatous neurocristoma of the uterus has been reported, but the source of pigment was considered to be melanocytic blue nevus cells within the leiomyoma not pigmented smooth muscle cells. Do renal angiomyolipoma cells make tyrosinase but fail to make melanin? If renal angiomyolipoma cells are truly related to melanocytes, why do smooth muscle or modified smooth muscle cells contain melanosomes? These and other questions remain to be elucidated about this distinctive tumor.

References


57. Martin PC, Pultizer D, Reed R. Pigmented myomatous neurocristoma of the uterus [see comments]. Arch Pathol Lab Med. 1989;113:1291–1295.