

Cathepsin K expression in the spectrum of perivascular epithelioid cell (PEC) lesions of the kidney

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The perivascular epithelioid cell (PEC) is a unique cell type coexpressing contractile proteins (mainly α -smooth muscle actin), melanocytic markers, including microphthalmia-associated transcription factor (MITF), and estrogen and progesterone receptors. It is constantly present in a group of tumors called PEComas. Renal PEComas include the common angiomyolipoma as well as less common lesions such as microscopic angiomyolipoma, intraglomerular lesions, angiomyolipoma with epithelial cysts, epithelioid angiomyolipoma, oncocyoma-like angiomyolipoma and lymphangioliomyomatosis of the renal sinus. It has been demonstrated that most of these lesions are determined by mutations affecting genes of the tuberous sclerosis complex, *tuberous sclerosis 1 (TSC1)* and *tuberous sclerosis 2 (TSC2)*, with eventual deregulation of the RHEB/MTOR/RPS6KB2 pathway, and it has been observed that some PEComas regressed during sirolimus therapy, an MTOR inhibitor. Recently, overexpression of MITF has been related to the expression of the papain-like cysteine protease cathepsin K in osteoclasts where it has inhibited MTOR. The aim of this study is to evaluate cathepsin K immunohistochemically in the entire spectrum of PEComa lesions in the kidney. The study population consisted of 84 renal PEComa lesions, including 5 composed predominantly of fat (lipoma-like angiomyolipoma), 15 almost exclusively composed of spindle-shaped smooth muscle cells (leiomyoma-like angiomyolipoma) and 31 common angiomyolipomas composed of a mixture of fat, spindle and epithelioid smooth muscle cells, and abnormal thick-walled blood vessels, 15 microscopic angiomyolipomas, 5 intraglomerular lesions, 2 oncocyoma-like angiomyolipomas, 8 epithelioid angiomyolipomas, 2 angiomyolipomas with epithelial cysts and 1 example of lymphangioliomyomatosis of the renal sinus. In all of the renal PEComas, cathepsin K was found to be constantly and strongly expressed and seems to be a more powerful marker than other commonly used markers for their identification, especially to confirm the diagnosis on needle biopsies.

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The perivascular epithelioid cell (PEC) is a unique cell type coexpressing contractile proteins (mainly α -smooth muscle actin (α -SMA)), melanocytic markers, including microphthalmia-associated transcription factor (MITF), and estrogen and progester-

one receptors. The PEC is constantly present in a group of tumors called PEComas including angiomyolipoma, clear-cell 'sugar' tumor of the lung and extrapulmonary sites, lymphangioliomyomatosis, clear-cell myomelanocytic tumor of the falciiform ligament and ligamentum teres, and rare clear-cell tumors of other anatomical sites. PEComas of the kidney include the common angiomyolipoma, microscopic angiomyolipoma, intraglomerular lesions, angiomyolipoma with epithelial cysts, epithelioid angiomyolipoma (pure epithelioid PEComa),

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oncocyoma-like angiomyolipoma and lymphangioliomyomatosis of the renal sinus. Angiomyolipoma is the most common mesenchymal neoplasm of the kidney and occurs both sporadically and in patients with tuberous sclerosis, a syndrome caused by losses of *tuberous sclerosis 1* (*TSC1*; 9q34) or *tuberous sclerosis 2* (*TSC2*; 16p13.3) and characterized by mental retardation, seizures and cellular proliferations.^{1,2} The pathogenesis of angiomyolipoma is determined by mutations affecting *TSC* genes, with eventual deregulation of the RHEB/MTOR/RPS6KB2 pathway.³ It has been observed that angiomyolipomas regressed somewhat during sirolimus therapy, an MTOR inhibitor, but tended to increase in volume after the therapy was stopped.⁴

Histologically, the common angiomyolipoma is characterized by the presence of a variable mixture of fat, spindle and epithelioid smooth muscle cells, and abnormal thick-walled blood vessels. Occasionally, adipocytes or spindle-shaped smooth muscle cells predominate and other elements are only sparsely represented (lipoma-like and leiomyoma-like angiomyolipoma). The common angiomyolipoma is benign.⁵ Sarcomatous transformation developing in the common angiomyolipoma has been reported, but is very rare.^{6,7} In the 1990s, a tumor called epithelioid angiomyolipoma was discovered.^{8–10} It is composed purely of epithelioid and stubby spindle cells arranged in sheets and is characterized by the absence or extreme paucity of both adipocytes and abnormal blood vessels. The cytoplasm of the neoplastic cells in these tumors varies from eosinophilic to clear and some of these cells can superficially resemble ganglion cells. Epithelioid angiomyolipoma (pure epithelioid PEComa) resembles high-grade or sarcomatoid renal cell carcinoma and has been misdiagnosed as carcinoma by frozen section, fine needle aspiration cytology and permanent section examinations. Epithelioid angiomyolipoma (pure epithelioid PEComa) can recur locally, metastasize and cause death.^{11–13} Epithelioid angiomyolipomas (pure epithelioid PEComa) have been found in patients with and without evidence of tuberous sclerosis and in the *TSC2/PKD1* contiguous gene syndrome. Loss of heterozygosity of *TSC2* has been reported in a few cases of sporadic epithelioid angiomyolipoma.^{9,14,15} Tumors composed of a homogeneous population of polygonal cells with deeply eosinophilic cytoplasm have been identified in patients with and without tuberous sclerosis and reported as oncocyoma-like angiomyolipoma.¹⁶ Angiomyolipoma containing epithelial cysts is the most recently described variant. It is a smooth muscle-predominant tumor in which are embedded epithelium-lined cysts with a compact subepithelial 'cambium-like' layer of stromal cells positive for HMB-45, estrogen and progesterone receptors.^{17–19} Lymphangioliomyomatosis of the renal sinus is a plaque-like proliferation in the wall of the renal pelvis. All three cases reported to date

also had renal angiomyolipomas, but in only one of them was an association with pulmonary lymphangioliomyomatosis observed.^{14,20}

Based on the recent demonstration that MTOR inhibitors can regulate major functional activities of osteoclasts, including the expression of cathepsin K, and on the demonstration that the expression of cathepsin K in osteoclasts is regulated by MITF,²¹ we investigated PEComa lesions of the kidney for the expression of cathepsin K, a papain-like cysteine protease with high matrix-degrading activity, to better understand the molecular mechanisms involved in their pathogenesis and to evaluate its diagnostic usefulness in comparison with other commonly used markers.

Materials and methods

Study Population

We investigated 84 renal PEComa lesions retrieved from the files of the Department of Pathology and Diagnostics of the University of Verona. These included 51 renal angiomyolipomas of variable size, including 5 composed predominantly of fat (lipoma-like angiomyolipoma), 15 almost exclusively composed of spindle-shaped smooth muscle cells (leiomyoma-like angiomyolipoma) and 31 composed of a mixture of fat, spindle and epithelioid smooth muscle cells, and abnormal thick-walled blood vessels, 15 microscopic angiomyolipomas, 5 intraglomerular lesions, 2 oncocyoma-like angiomyolipomas, 8 epithelioid angiomyolipomas (pure epithelioid PEComas), 2 angiomyolipomas with epithelial cysts and 1 example of lymphangioliomyomatosis of the renal pelvis. Some of these lesions have been included in previous studies.^{9,10,14,16,22} We have additionally tested three postoperative needle biopsies of common renal angiomyolipomas.

Control cases included 350 clear-cell renal cell carcinomas, 80 papillary renal cell carcinomas, 50 chromophobe renal cell carcinomas, 50 renal oncocytomas and the normal renal parenchyma of kidneys harboring the neoplasms.

Immunohistochemical Staining and Antibodies

All tissue samples were formalin fixed and paraffin embedded according to standard methods. Sections from tissue blocks of PEComas and control cases were stained immunohistochemically with the following antibodies: α -SMA (clone 2A4, Dako, Glostrup, Denmark), HMB45 (Dako); MITF (clone D5, Neomarkers/LabVision, Fremont, CA, USA), melan-A (clone A103, Biogenex, San Ramon, CA, USA), CD68 (clone PG-M1, Dako Glostrup, Denmark), CD68 (clone KP1, Dako) and two different mouse monoclonal antibodies recognizing human cathepsin K (clone CK4, Novocastra, Newcastle, UK;

and clone 3F9, Abcam, Cambridge, UK). Heat-induced antigen retrieval for cathepsin K was performed using a microwave oven and 0.01 mol/l of citrate buffer, pH 6.0, for 30 min. All samples were processed using a sensitive 'Bond polymer Refine' detection system in an automated Bond immunohistochemistry instrument (Vision-Biosystem, Menarini, Florence, Italy). Sections incubated without the primary antibody served as negative controls.

Results

The results are summarized in Table 1.

Common Angiomyolipoma

In all examples of common renal angiomyolipoma, strong and diffuse cathepsin K expression was demonstrated, clearly distinguishing the neoplastic lesion from the adjacent normal renal parenchyma regardless of the size of the tumors or their composition (Figure 1a). Both cathepsin K-specific antibodies showed comparable intensities of staining. All types of PEComa cells including spindle-shaped and epithelioid smooth muscle and adipocyte-like cells were strongly and homogeneously immunoreactive for cathepsin K (Figure 1b). The endothelium and the walls of the vessels present in the tumors were unstained with the antibodies to cathepsin K. The α -SMA, HMB45 (Figure 1c), MITF, melan-A²³ and CD68 (both the clones tested: PG-M1 and KP1; Figures 1d and e) were expressed in common angiomyolipomas as previously described.^{22,24,25} All three angiomyolipoma needle biopsies showed focal HMB-45 immunoreactivity and strong and diffuse staining for cathepsin K (both antibodies; Figures 2a–c).

Microscopic Angiomyolipoma and Intraglomerular Lesions

All of the microscopic angiomyolipomas displayed strongly positive reactions for cathepsin K, which was easy identification of even the smallest lesions arising in the renal parenchyma (Figures 3a and b). Both antibodies to cathepsin K showed comparable reactions. The α -SMA, HMB45, MITF, melan-A and CD68 (tested with both the clones) were also found to be expressed.

Two intraglomerular lesions consisted of minute nodules composed of a few adipocytes and epithelioid smooth muscle cells within the slightly compressed glomerular capillary tuft and without any attachment to Bowman's capsule, whereas the other three intraglomerular lesions were exclusively composed of epithelioid smooth muscle cells (Figure 3c). In the first two intraglomerular

lesions, reactions for α -SMA, HMB45, MITF and melan-A were constantly negative; in the latter three intraglomerular lesions, only the reaction for α -SMA was focally positive. Finally, cathepsin K was expressed in all of the intraglomerular lesions (Figure 3d). In the sections stained for CD68, the intraglomerular lesions were no longer present.

Although many (13 out of 15) of the microscopic angiomyolipomas and some (3 out of 5) of the intraglomerular lesions were recognizable in routine sections, a few of them were revealed by immunohistochemical staining for cathepsin K. Specifically, the two microscopic angiomyolipoma unrecognized in routine sections were composed of a few spindle-shaped smooth muscle cells in the renal interstitium.

Oncocytoma-Like Angiomyolipoma

The two oncocytoma-like angiomyolipomas stained strongly and diffusely positively with both antibodies to cathepsin K. Approximately 10% of the cells had a diffuse cytoplasmic positive reaction for HMB45 and melan-A. The reaction with antibody to α -SMA showed staining at the cytoplasmic membrane in ~10 and 70% of the neoplastic cells of the two tumors. There was a positive nuclear reaction for MITF in ~30% of the cells in one tumor, but the other was negative. Finally, CD68 was detected in 15 and 2% (PG-M1) and in 60 and 2% (KP1) of cells in the two tumors.

Angiomyolipoma with Epithelial Cysts

Both angiomyolipomas with epithelial cysts showed a composite aspect, solid and cystic. In one tumor the cyst was solitary (Figures 4a and b) whereas the other tumor contained multiple cysts (Figure 5a). Microscopically, angiomyolipoma with epithelial cysts is composed of three components: the cysts lined by cuboidal to hobnail epithelial cells, a compact subepithelial 'cambium-like' layer of stromal cells (Figures 5b and 6a) that gave positive reactions for HMB45, melan-A, MITF (Figure 6b) and SMA, and a solid extra cystic component with the morphologic features of a leiomyoma-like angiomyolipoma with a concordant immunophenotype. Both the stromal cells of the 'cambium-like' layer and the smooth muscle leiomyoma-like angiomyolipoma showed strong expression of cathepsin K demonstrated with both antibodies (Figure 6c). In case 1, CD68 tested with both the clones was expressed focally (2%) in the leiomyoma-like and the epithelioid angiomyolipomas. In case 2, CD68 was expressed in 10% (PG-M1) and in 30% (KP1) of the cells in the leiomyoma-like component and in the 'cambium-like' layer (Figure 6d).

Table 1 Immunohistochemical results

	<i>Cathepsin K</i>	<i>HMB45</i>	<i>CD68 (PG-M1)</i>	<i>CD68 (KP1)</i>	<i>MITF</i>	<i>Melan-A</i>	α - <i>SMA</i>
<i>Common angiomyolipoma</i>							
Case 1	+ (90%)	+ (10%)	+ (10%)	+ (50%)	+ (60%)	+ (30%)	+ (60%)
Case 2	+ (80%)	+ (10%)	+ (20%)	+ (40%)	+ (30%)	+ (10%)	+ (40%)
Case 3	+ (90%)	+ (5%)	+ (10%)	+ (50%)	Negative	+ (50%)	+ (40%)
Case 4	+ (95%)	+ (30%)	+ (10%)	+ (50%)	Negative	+ (50%)	+ (50%)
Case 5	+ (85%)	+ (10%)	+ (5%)	+ (60%)	Negative	+ (10%)	+ (50%)
Case 6	+ (100%)	+ (5%)	+ (10%)	+ (45%)	+ (30%)	+ (90%)	+ (40%)
Case 7	+ (90%)	+ (5%)	+ (5%)	+ (50%)	+ (10%)	+ (80%)	+ (50%)
Case 8	+ (80%)	+ (10%)	+ (15%)	+ (55%)	Negative	+ (70%)	+ (55%)
Case 9	+ (90%)	+ (10%)	+ (10%)	+ (30%)	+ (5%)	+ (50%)	n.a.
Case 10	+ (90%)	+ (10%)	+ (20%)	+ (50%)	+ (20%)	+ (30%)	+ (70%)
Case 11	+ (90%)	+ (20%)	+ (15%)	+ (50%)	Negative	+ (70%)	+ (50%)
Case 12	+ (85%)	+ (5%)	n.a.	+ (45%)	Negative	+ (5%)	+ (70%)
Case 13	+ (90%)	+ (10%)	+ (10%)	+ (50%)	Negative	+ (20%)	+ (60%)
Case 14	+ (90%)	+ (10%)	+ (5%)	+ (60%)	Negative	+ (20%)	+ (60%)
Case 15	+ (100%)	+ (15%)	+ (10%)	+ (40%)	+ (80%)	n.a.	+ (40%)
Case 16	+ (90%)	+ (15%)	+ (10%)	+ (40%)	+ (10%)	+ (90%)	+ (70%)
Case 17	+ (85%)	+ (5%)	+ (5%)	+ (50%)	+ (40%)	+ (90%)	+ (50%)
Case 18	+ (90%)	Negative	+ (10%)	+ (30%)	Negative	+ (40%)	+ (30%)
Case 19	+ (80%)	+ (5%)	+ (15%)	+ (50%)	+ (10%)	+ (50%)	+ (60%)
Case 20	+ (85%)	+ (10%)	+ (10%)	+ (30%)	+ (60%)	+ (50%)	n.a.
Case 21	+ (90%)	+ (15%)	+ (10%)	+ (45%)	Negative	+ (70%)	+ (50%)
Case 22	+ (90%)	+ (10%)	+ (10%)	+ (50%)	Negative	+ (60%)	+ (70%)
Case 23	+ (90%)	+ (15%)	+ (15%)	+ (60%)	+ (10%)	+ (40%)	+ (60%)
Case 24	+ (80%)	+ (15%)	+ (20%)	+ (60%)	+ (20%)	+ (80%)	+ (50%)
Case 25	+ (90%)	+ (20%)	+ (10%)	n.a.	Negative	+ (5%)	+ (70%)
Case 26	+ (100%)	+ (5%)	+ (10%)	+ (50%)	Negative	+ (40%)	+ (50%)
Case 27	+ (90%)	+ (5%)	+ (15%)	+ (50%)	Negative	+ (30%)	+ (50%)
Case 28	+ (90%)	+ (10%)	+ (5%)	+ (55%)	Negative	+ (30%)	+ (40%)
Case 29	+ (90%)	+ (10%)	+ (10%)	+ (45%)	Negative	+ (10%)	+ (70%)
Case 30	+ (100%)	+ (20%)	+ (20%)	+ (70%)	+ (30%)	+ (20%)	+ (30%)
Case 31	+ (80%)	+ (10%)	+ (10%)	+ (40%)	+ (50%)	+ (60%)	+ (60%)
<i>Common angiomyolipoma (biopsy)</i>							
Case 1	+ (90%)	+ (10%)	n.a.	n.a.	n.a.	n.a.	n.a.
Case 2	+ (80%)	+ (10%)	n.a.	n.a.	n.a.	n.a.	n.a.
Case 3	+ (90%)	+ (5%)	n.a.	n.a.	n.a.	n.a.	n.a.
<i>Lipoma-like angiomyolipoma</i>							
Case 1	+ (90%)	+ (10%)	+ (10%)	+ (50%)	+ (10%)	+ (40%)	+ (10%)
Case 2	+ (85%)	+ (10%)	+ (5%)	+ (40%)	Negative	+ (50%)	+ (10%)
Case 3	+ (90%)	n.a.	+ (10%)	+ (30%)	Negative	+ (60%)	+ (20%)
Case 4	+ (100%)	+ (10%)	+ (20%)	+ (50%)	+ 10%	+ (20%)	+ (5%)
Case 5	+ (90%)	+ (5%)	+ (15%)	+ (60%)	Negative	+ (10%)	+ (10%)
<i>Leiomyoma-like angiomyolipoma</i>							
Case 1	+ (90%)	+ (10%)	+ (10%)	+ (50%)	+ (10%)	+ (60%)	+ (70%)
Case 2	+ (90%)	+ (5%)	+ (20%)	+ (60%)	Negative	+ (90%)	+ (50%)
Case 3	+ (80%)	+ (10%)	+ (5%)	+ (60%)	Negative	+ (40%)	+ (80%)
Case 4	+ (100%)	n.a.	+ (10%)	+ (40%)	+ (40%)	+ (10%)	+ (80%)
Case 5	+ (90%)	+ (10%)	+ (10%)	+ (50%)	Negative	Negative	+ (60%)
Case 6	+ (85%)	Negative	+ (10%)	+ (45%)	Negative	+ (70%)	+ (70%)
Case 7	+ (90%)	+ (10%)	+ (20%)	+ (50%)	Negative	n.a.	+ (80%)
Case 8	+ (100%)	+ (5%)	+ (10%)	+ (50%)	+ (20%)	+ (40%)	+ (60%)
Case 9	+ (80%)	+ (5%)	+ (15%)	+ (55%)	+ (50%)	+ (80%)	+ (60%)
Case 10	+ (90%)	+ (10%)	n.a.	+ (45%)	Negative	+ (80%)	+ (50%)
Case 11	+ (100%)	+ (15%)	+ (10%)	+ (70%)	+ (10%)	+ (20%)	n.a.
Case 12	+ (90%)	+ (20%)	+ (15%)	+ (50%)	Negative	+ (40%)	+ (80%)
Case 13	+ (100%)	+ (10%)	+ (15%)	+ (40%)	n.a.	+ (90%)	+ (50%)
Case 14	+ (80%)	Negative	+ (5%)	+ (40%)	+ (60%)	+ (50%)	+ (80%)
Case 15	+ (90%)	+ (15%)	+ (20%)	+ (50%)	+ (30%)	+ (5%)	+ (70%)
<i>Microscopic angiomyolipoma</i>							
Case 1	+ (100%)	+ (10%)	+ (20%)	+ (50%)	+ (20%)	+ (10%)	+ (40%)
Case 2	+ (90%)	+ (10%)	+ (5%)	+ (80%)	+ (10%)	+ (40%)	+ (70%)
Case 3	+ (90%)	+ (10%)	+ (10%)	+ (50%)	+ (60%)	+ (30%)	+ (30%)
Case 4	+ (85%)	+ (5%)	+ (10%)	+ (50%)	Negative	+ (5%)	+ (80%)
Case 5	+ (100%)	+ (5%)	+ (10%)	+ (60%)	Negative	+ (90%)	n.a.
Case 6	+ (100%)	+ (10%)	+ (15%)	+ (60%)	+ (10%)	+ (90%)	+ (50%)
Case 7	+ (90%)	+ (10%)	+ (15%)	+ (70%)	Negative	+ (50%)	+ (50%)
Case 8	+ (90%)	+ (15%)	+ (20%)	+ (50%)	+ (5%)	+ (80%)	+ (30%)

Table 1 Continued

	<i>Cathepsin K</i>	<i>HMB45</i>	<i>CD68 (PG-M1)</i>	<i>CD68 (KP1)</i>	<i>MITF</i>	<i>Melan-A</i>	α - <i>SMA</i>
Case 9	+ (90%)	+ (5%)	+ (20%)	+ (55%)	+ (10%)	+ (30%)	+ (70%)
Case 10	+ (85%)	+ (15%)	+ (20%)	+ (60%)	Negative	+ (80%)	+ (30%)
Case 11	+ (100%)	+ (10%)	+ (5%)	+ (50%)	Negative	+ (20%)	+ (40%)
Case 12	+ (90%)	+ (5%)	+ (15%)	n.a.	+ (40%)	+ (5%)	+ (70%)
Case 13	+ (100%)	+ (20%)	+ (10%)	+ (50%)	+ (5%)	+ (10%)	+ (50%)
Case 14	+ (100%)	+ (5%)	+ (20%)	+ (45%)	Negative	+ (90%)	+ (30%)
Case 15	+ (90%)	Negative	+ (10%)	+ (40%)	+ (30%)	+ (70%)	+ (30%)
<i>Intraglomerular lesions</i>							
Case 1	+ (70%)	Negative	n.a.	n.a.	Negative	Negative	Negative
Case 2	+ (60%)	Negative	n.a.	n.a.	Negative	Negative	Negative
Case 3	+ (70%)	Negative	n.a.	n.a.	Negative	Negative	+ (5%)
Case 4	+ (90%)	Negative	n.a.	n.a.	Negative	Negative	+ (5%)
Case 5	+ (90%)	Negative	n.a.	n.a.	Negative	Negative	+ (5%)
<i>Oncocytoma-like angiomyolipomas</i>							
Case 1	+ (90%)	+ (10%)	+ (15%)	+ (60%)	+ (30%)	+ (10%)	+ (10%)
Case 2	+ (90%)	+ (10%)	+ (2%)	+ (2%)	Negative	+ (10%)	+ (70%)
<i>Angiomyolipomas with epithelial cysts</i>							
Case 1							
Leiomyoma-like AML	+ (90%)	+ (20%)	+ (2%)	+ (2%)	+ (10%)	+ (40%)	+ (30%)
Epithelioid AML	+ (90%)	+ (10%)	+ (2%)	+ (2%)	Negative	+ (90%)	+ (40%)
Epithelium of the cysts	Negative	Negative	Negative	Negative	Negative	Negative	Negative
'Cambium-like' layer	+ (90%)	+ (5%)	Negative	Negative	+ (50%)	+ (5%)	+ (30%)
Case 2							
Leiomyoma-like AML	+ (90%)	+ (40%)	+ (10%)	+ (30%)	+ (40%)	+ (30%)	+ (60%)
Epithelium of the cysts	Negative	Negative	Negative	Negative	Negative	Negative	Negative
'Cambium-like' layer	+ (90%)	+ (40%)	+ (10%)	+ (30%)	+ (30%)	+ (50%)	+ (40%)
<i>Epithelioid angiomyolipomas</i>							
Case 1	+ (90%)	+ (70%)	+ (40%)	+ (50%)	+ (30%)	+ (40%)	+ (10%)
Case 2	+ (100%)	+ (80%)	+ (50%)	+ (90%)	+ (10%)	+ (30%)	+ (10%)
Case 3	+ (95%)	+ (80%)	+ (80%)	+ (50%)	+ (40%)	+ (10%)	Negative
Case 4	+ (90%)	+ (100%)	+ (60%)	+ (80%)	Negative	+ (5%)	+ (20%)
Case 5	+ (100%)	+ (80%)	+ (80%)	+ (60%)	Negative	+ (90%)	+ (5%)
Case 6	+ (100%)	+ (40%)	+ (50%)	+ (80%)	+ (80%)	+ (50%)	Negative
Case 7	+ (95%)	+ (80%)	+ (40%)	+ (50%)	Negative	+ (50%)	Negative
Case 8	+ (90%)	+ (80%)	+ (70%)	+ (60%)	+ (50%)	+ (30%)	Negative
<i>Lymphangioleiomyomatosis of the renal sinus</i>							
Case 1	+ (90%)	+ (80%)	Negative	+ (90%)	+ (30%)	+ (50%)	+ (60%)

n.a.: not available; AML: angiomyolipoma; MITF: microphthalmia-associated transcription factor; α -SMA: α -smooth muscle actin.

Epithelioid Angiomyolipoma (Pure Epithelioid PEComa)

All of the epithelioid angiomyolipomas (pure epithelioid PEComa; Figures 4a and b, arrow), those occurring in patients without tuberous sclerosis as well as those in patients with tuberous sclerosis, were composed of densely packed large cells ranging from spindle cells with mild nuclear atypia to strikingly atypical polygonal cells mixed with a variable number of multinucleated cells. All of them showed a diffuse strongly positive reaction for cathepsin K (Figure 4c) with both antibodies. HMB45 and melan-A were similarly diffusely expressed, but only a minority of cells reacted with antibodies to α -SMA (2 out of 8 tumors) and MITF in 5 out of 8 tumors, except for one tumor that gave a positive reaction in 80% of the cells. Finally, the reaction for CD68 was strongly positive in a percentage of neoplastic cells that ranged from 40 to 80% (PG-M1) and 50 to 90% (KP1; Figure 6).

Lymphangioleiomyomatosis of the Renal Sinus

This lesion consisted of a plaque-like proliferation of spindle-shaped and epithelioid smooth muscle cells containing branching slit-like vascular channels (Figure 7a). This lesion showed a strong and diffusely positive reaction for cathepsin K (Figure 7b). The reactions for SMA, melan-A, HMB45 and CD68 (clone KP1) were positive, but the reactions were negative for CD68 with clone PG-M1.

Control Samples

None of the clear-cell renal cell carcinomas, papillary renal cell carcinomas, chromophobe renal cell carcinomas, renal oncocytomas or normal renal parenchyma gave a positive reaction with either of the antibodies to cathepsin K, except for two oncocytomas that displayed focal but unequivocal positive reactions.

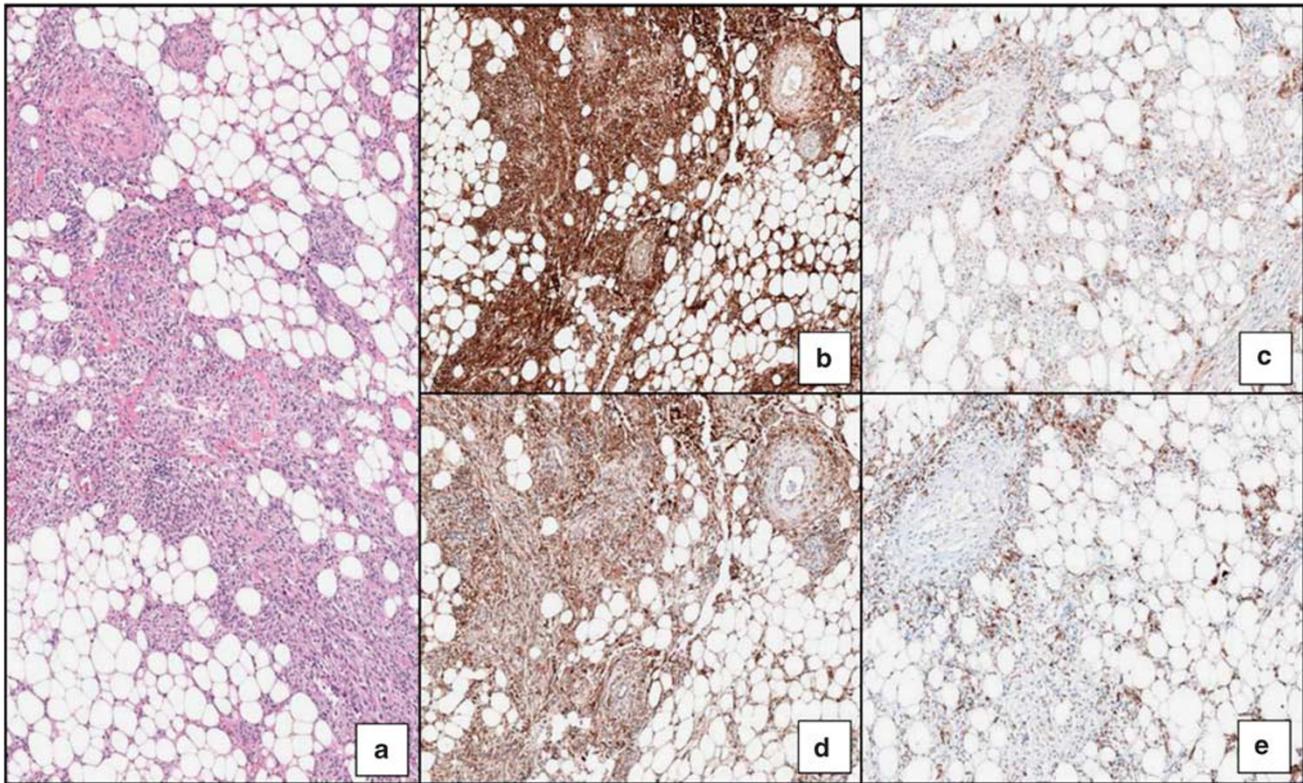


Figure 1 (a) Common angiomyolipoma composed of spindle, epithelioid smooth muscle and adipocyte-like cells. (b) Strong and diffuse cathepsin K expression in all types of cells including spindle cells, epithelioid smooth muscle cells and adipocyte-like cells. (c) HMB45 expression in a subset of PEComa cells of a common angiomyolipoma. (d, e) CD68 (antibody KP1) and CD68 (antibody PG-M1) expression in common angiomyolipoma.

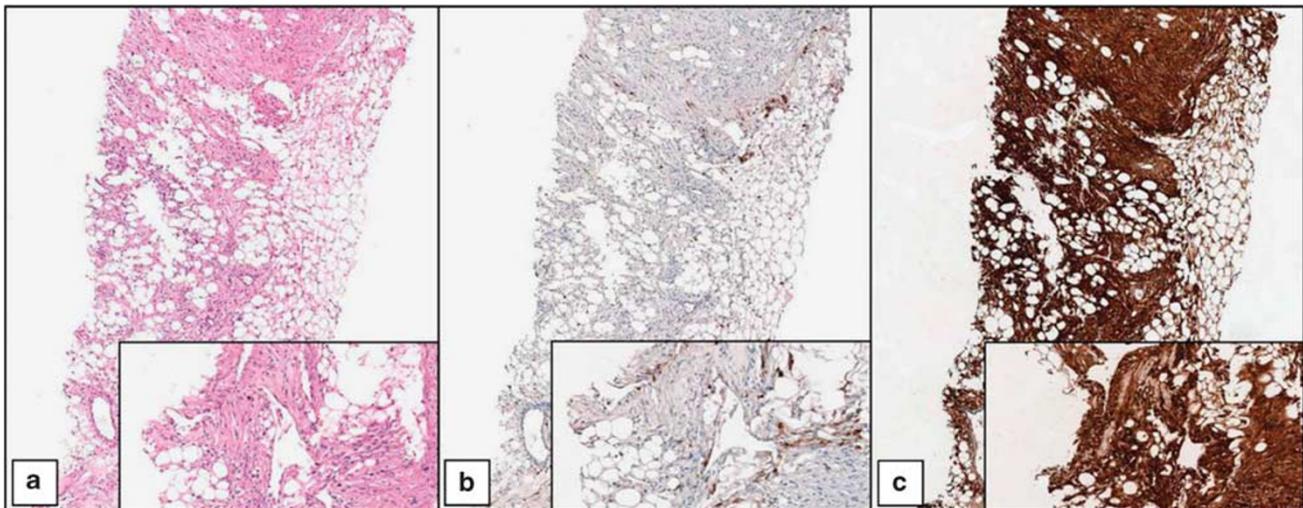


Figure 2 (a) Bench biopsy of a common angiomyolipoma. (b) Scattered HMB45-positive PEComa cells. (c) Diffuse and strong immunoeexpression of cathepsin K.

Discussion

In this study we found that cathepsin K is expressed in the entire spectrum of PEC lesions of the kidney: common angiomyolipoma, including ones with extreme predominance of fat and spindle-shaped

smooth muscle cells, microscopic angiomyolipomas, intraglomerular lesions, angiomyolipoma with epithelial cysts, oncocytoma-like angiomyolipoma, epithelioid angiomyolipoma (pure epithelioid PEComa) and lymphangioliomyomatosis of the renal sinus.

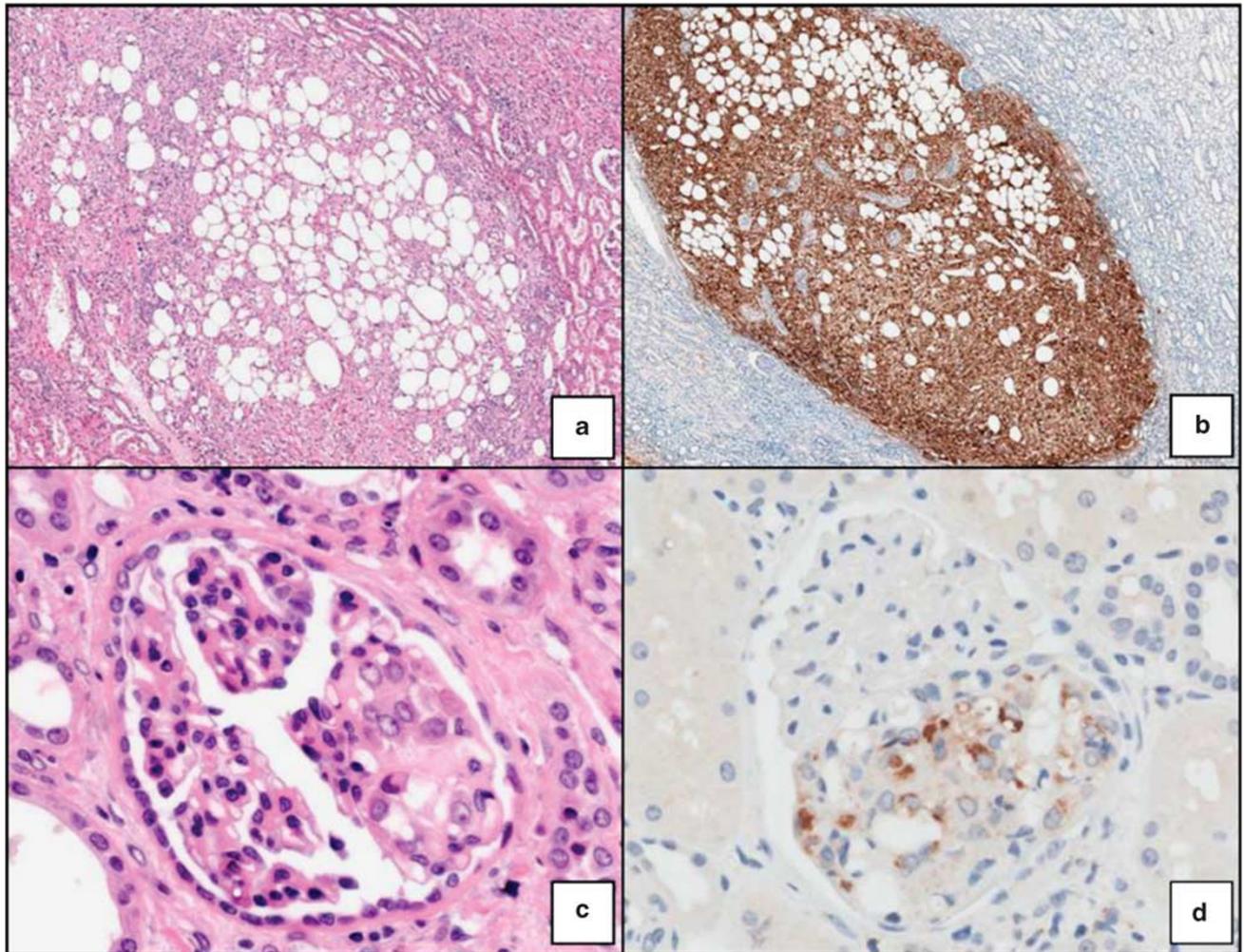


Figure 3 (a, b) Microscopic angiomyolipoma displaying strong cathepsin K positivity. (c, d) Intraglomerular lesion exclusively composed of epithelioid smooth muscle cells expressing cathepsin K.

The common angiomyolipoma, which is composed of a variable mixture of fat, spindle and epithelioid smooth muscle cells and abnormal thick-walled blood vessels, represents the prototype of the capacity of PECs to modulate their morphology. PECs can show smooth muscle differentiation with a spindle shape and a stronger immunoreactivity for actin than for HMB45 or can have an epithelioid shape with a strong expression of HMB45 and a slight, if any, expression of actin. PECs can also develop lipid-filled vacuoles, acquiring the features of adipocytes. Common angiomyolipomas typically contain more than one cell type; however, in some tumors one cell type predominates and these angiomyolipomas are consequently named lipoma-like or leiomyoma-like angiomyolipoma.² In this study we have demonstrated the constant finding of strong cathepsin K immunoreactivity in the spindle and epithelioid smooth muscle-like and adipocyte-like cells in renal angiomyolipoma. Moreover, this finding is according to

the previously described role of cathepsin K in adipocyte differentiation.²⁶ Finally, the strong and diffuse cathepsin K immunoreactivity in angiomyolipoma suggests the utility of adding this marker to the immunophenotypic profile by which to test percutaneous renal biopsies obtained for the diagnosis of the small masses of the kidney. In this setting, cathepsin K, which we have shown to be diffusely positive, can be more useful for the diagnosis of angiomyolipoma than HMB45, which is also very specific but often displays just a focal positive reaction.

Angiomyolipoma can occur sporadically, most frequently in a woman during her fourth to sixth decades of life, or in patients with tuberous sclerosis. In patients with tuberous sclerosis, the angiomyolipomas are usually multiple and variable in size. The smallest tumors are the microscopic angiomyolipomas and the intraglomerular lesions, which can be subtle and difficult to identify.⁵ For this reason, immunohistochemistry for cathepsin K

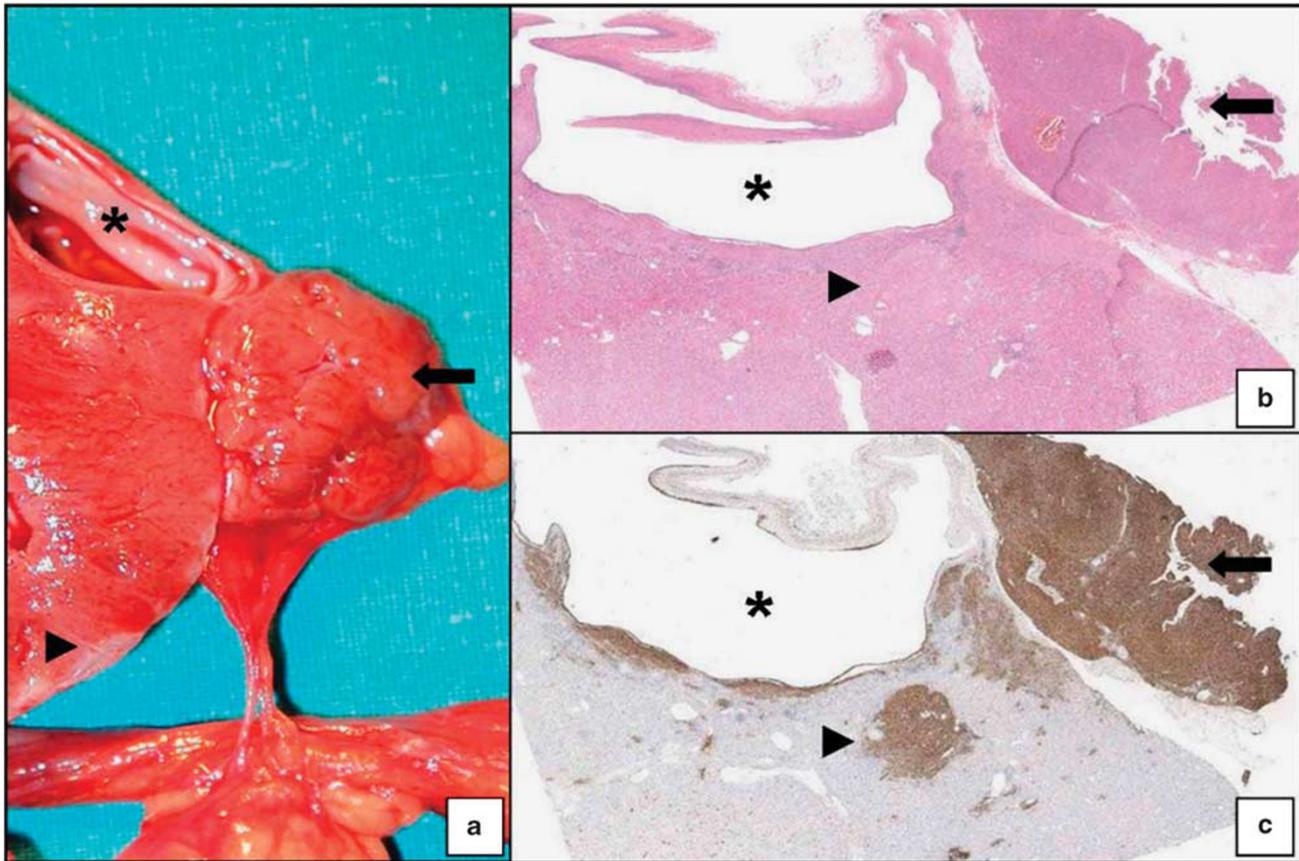


Figure 4 (a) Gross features of a kidney presenting simultaneously an angiomyolipoma with a single epithelial cyst (asterisk), an epithelioid angiomyolipoma (pure epithelioid PEComa; arrow), multiple microscopic angiomyolipomas (arrow head) and numerous intraglomerular lesions. (b) H&E section including all the PEComas involving this kidney: angiomyolipoma with a single epithelial cyst (asterisk), epithelioid angiomyolipoma (pure epithelioid PEComa; arrow) and microscopic angiomyolipoma (arrow head). (c) Cathepsin K is strongly expressed in the compact subepithelial 'cambium-like' layer of underlying epithelial cyst (asterisk), stains diffusely the epithelioid angiomyolipoma (pure epithelioid PEComa; arrow) and highlights even the smallest PEComas present in the renal parenchyma (arrow head).

can be useful to detect these microscopic lesions and to suggest the possibility of a diagnosis of tuberous sclerosis.

The demonstration of strong cathepsin K expression in angiomyolipoma can contribute to explain some features of these tumors, in particular their propensity for massive and spontaneous bleeding that can lead to the formation of voluminous retroperitoneal hematomas and hypovolemic shock (Wunderlich's syndrome).⁵ Although the propensity for massive bleeding can more likely be related to the rich vascular network of those tumors, cathepsin K is a papain-like cysteine protease with high matrix-degrading activity on collagen and elastin,²⁷ and this activity can significantly contribute to the progressive structural damage and remodeling of the walls of the abnormal blood vessels commonly found in angiomyolipomas.

Epithelioid angiomyolipoma (pure epithelioid PEComa) is a neoplasm included in the current WHO classification.²⁸ The strong, diffuse and constant immunohistochemical detection of cathepsin

K in epithelioid angiomyolipoma (pure epithelioid PEComa) and negative reactions for cathepsin K in the common types of renal cell carcinoma suggests the utility of immunohistochemistry for cathepsin K in the differential diagnosis of epithelioid angiomyolipoma (pure epithelioid PEComa) with renal cell carcinoma. Only the microphthalmia transcription factor/transcription factor E (MITF/TFE) family of translocation renal cell carcinomas (which comprise the majority of pediatric renal cell carcinomas but also occur in adults) have been shown to express cathepsin K. In a recent study, we have shown that all of the t(6;11) translocation carcinomas expressed cathepsin K as did 6 of 10 *TFE3* translocation carcinomas.²⁹ These carcinomas are characterized by specific chromosome aberrations involving the genes *transcription factor E3 (TFE3)* and *transcription factor EB (TFEB)* that cause the overexpression of the TFE3 fusion proteins or TFEB. A similar translocation involving *TFE3* gene has been recently reported in a subset of extrarenal PEComas, all of which also expressed cathepsin K.³⁰ Both TFE3 and

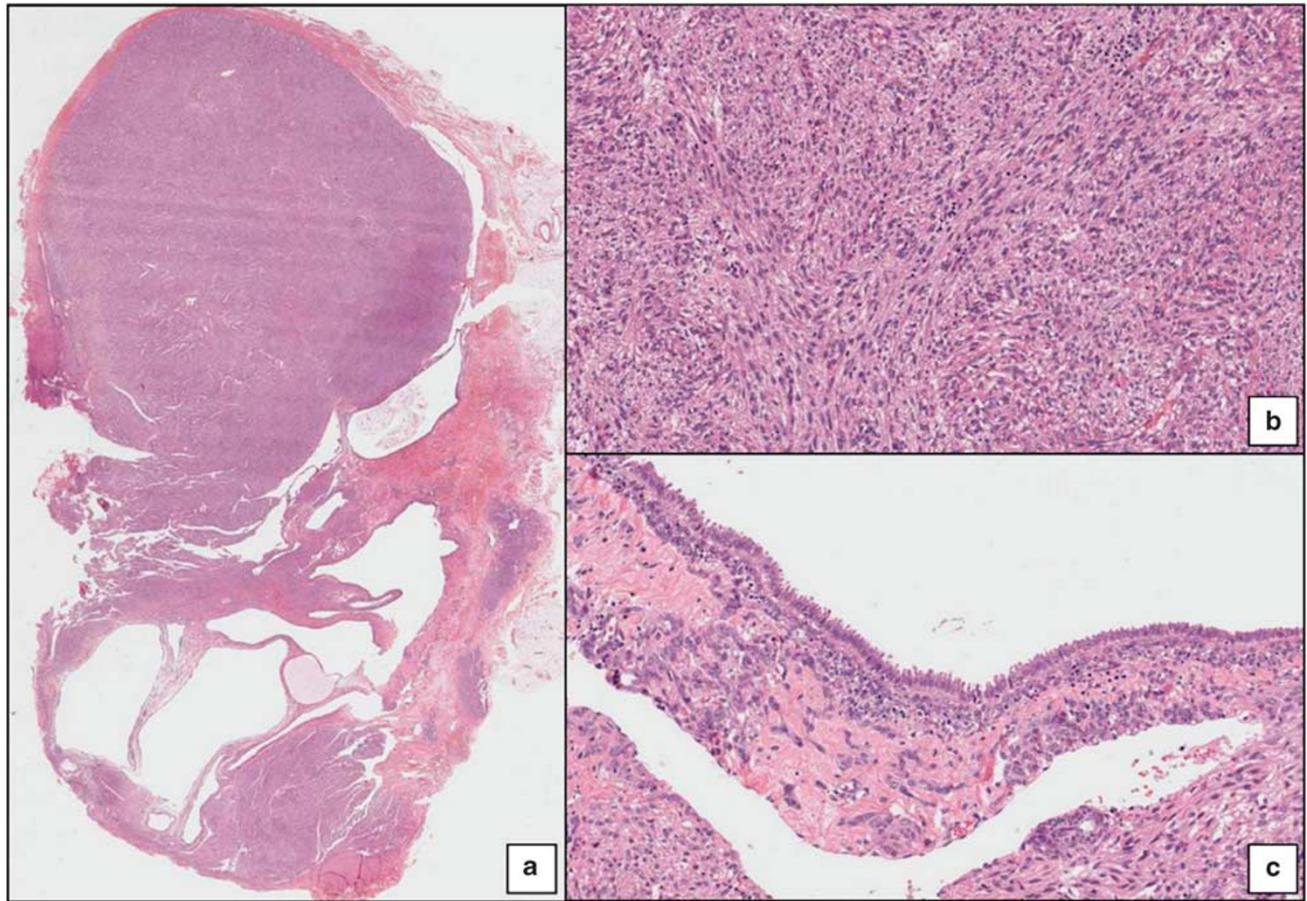


Figure 5 (a) Angiomyolipoma with multiple epithelial cysts. (b, c) This tumor is characterized by three components: a solid extracystic component with the morphologic feature of a leiomyoma-like angiomyolipoma, the epithelial cysts lined by cuboidal to hobnail cells and a compact subepithelial 'cambium-like' layer.

TFEB are closely related members of the MITF/TFE transcription factor family that also includes MITF, which has been previously found to be expressed in both lymphangiomyomatosis and angiomyolipomas.^{24,31} Recently, a gene network directing the maturation of melanosomes that is regulated by MITF, which is a possible heterodimerization partner and close homolog of TFEB, has been identified.^{32,33} All of these findings shed light on the common expression of cathepsin K and melanogenesis markers such as HMB45 and melan-A in both renal PEC proliferations and MITF/TFE family of renal translocation carcinomas, in particular tumors with the t(6;11) translocation. Although these findings are intriguing and highlight the possibility of some sort of relationship among these lesions,³⁰ the common expression of melanogenesis markers and numerous overlapping morphological features underline the diagnostic difficulty in distinguishing epithelioid angiomyolipoma from t(6;11) translocation renal cell carcinomas.^{12,30}

Another morphological differential diagnosis that can be difficult is between the extremely rare oncocytoma-like angiomyolipoma and the renal

oncocytoma.¹⁶ This differential is of interest because both oncocytomas and angiomyolipomas have been reported in the same kidney and because in patients with tuberous sclerosis there is some evidence that oncocytomas occur more frequently than in general population.³⁴ In our experience, the detection of HMB45 can be extremely focal in oncocytoma-like angiomyolipomas and, therefore, the strong and diffuse reaction for cathepsin K demonstrated in the present study can be diagnostically helpful.

None of the cells within the normal renal parenchyma expressed cathepsin K, neither in patients with tuberous sclerosis nor in patients without tuberous sclerosis. Thus, cathepsin K does not identify the precursor cell of PEComas that remains undiscovered. However, it seems likely that the aberrant expression of MITF in a mesenchymal precursor cell could result in a neoplasm (PEComa) that expresses cathepsin K and melanocytic markers and that a normal cell of origin expressing cathepsin K is lacking.

Finally, it is possible to speculate that MTOR inhibitors proposed as a new therapeutic option for PEC lesions^{4,35-37} can exert part of their activity by

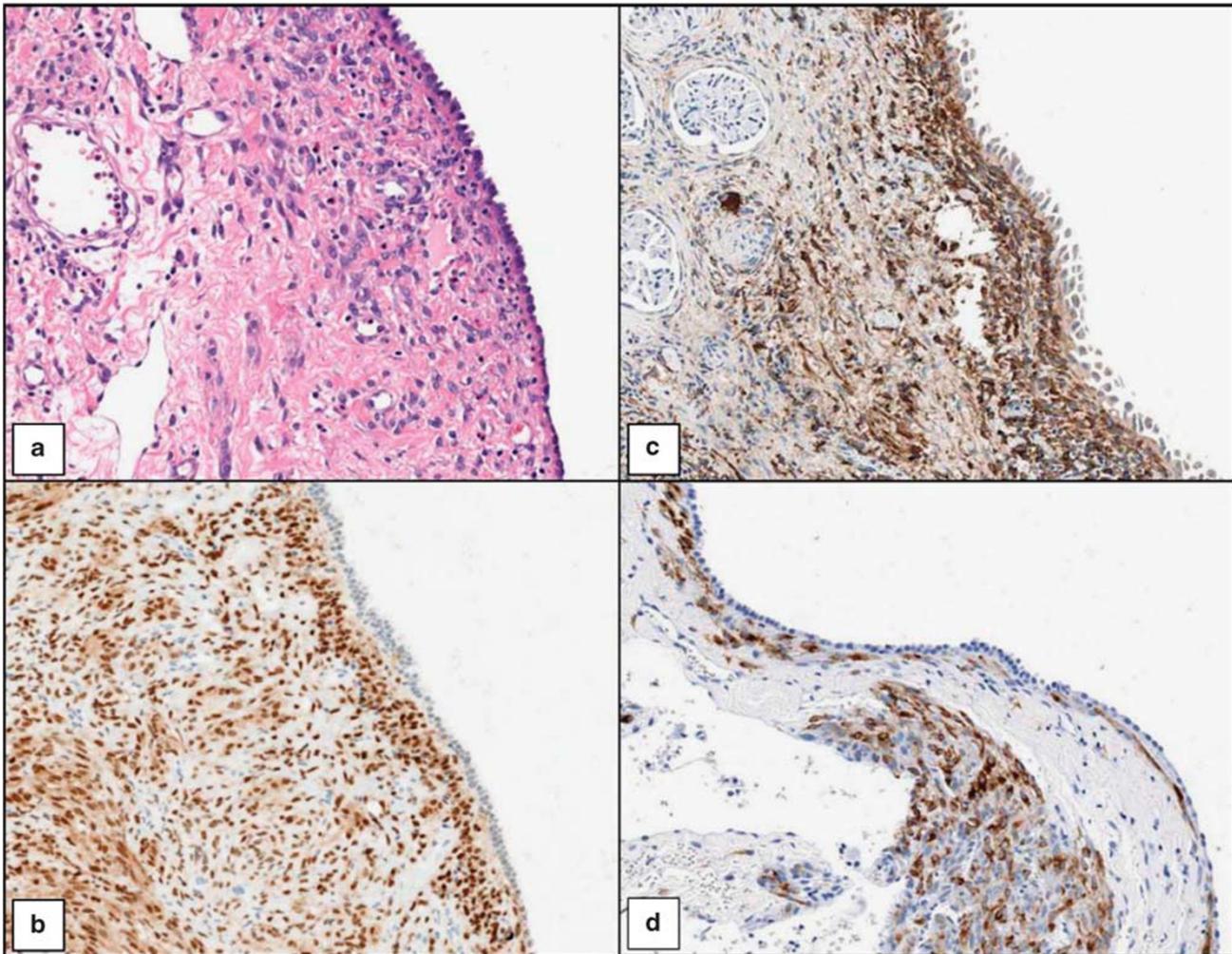


Figure 6 (a) Compact subepithelial 'cambium-like' layer of stromal cells in angiomyolipoma with epithelial cysts. (b-d) MITF, cathepsin K and CD68 (antibody PG-M1) immunoreactivities in the 'cambium-like' layer.

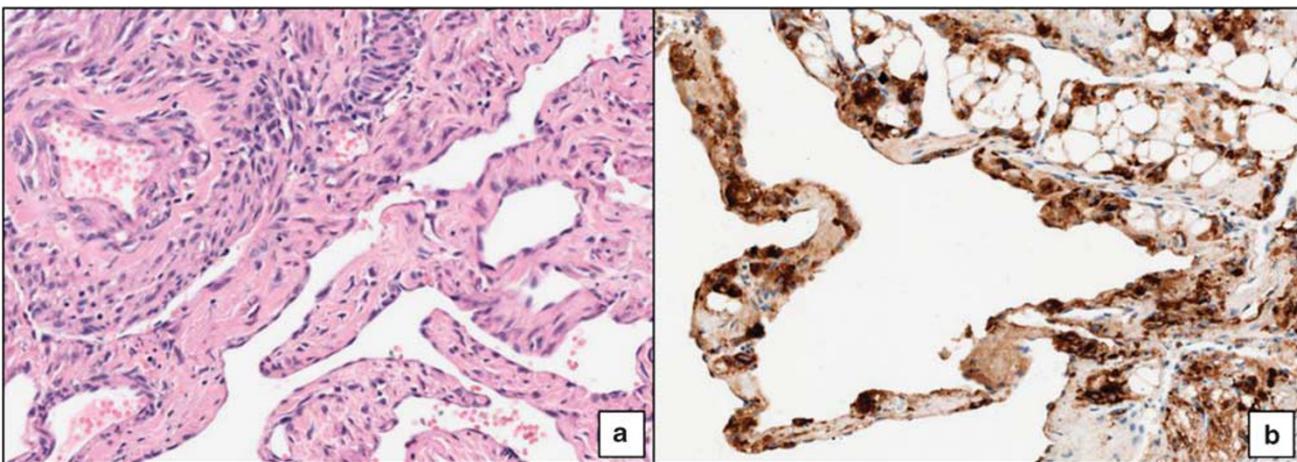


Figure 7 (a) Lymphangioliomyomatosis of the renal sinus consisted of a plaque-like proliferation of spindle and epithelioid smooth muscle cells containing branching slit-like vascular channels. (b) Strong and diffuse cathepsin K expression.

limiting the expression of cathepsin K. In favor of this interesting possibility is the observation that rapamycin and its analogs have efficacy in limiting the bone resorption mediated by osteoclastic

cathepsin K³⁸ and can interfere with the proliferation of melanoma,^{39,40} a tumor exhibiting MTOR pathway activation, as well as MITF and cathepsin K expression.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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