Molecular Pathology of Lymphangioleiomyomatosis and Other Perivascular Epithelioid Cell Tumors

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• ***Context.***—**Lymphangioleiomyomatosis (LAM) is a cystic lung disease that can be included in the wide group of proliferative lesions named PEComas (perivascular epithelioid cell tumors). These proliferative tumors are characterized by the coexpression of myogenic and melanogenesis-related markers. In all these lesions, genetic alterations related to the tuberous sclerosis complex (TSC) have been demonstrated. Striking improvements in the understanding of the genetic basis of this autosomal dominant genetic disease are coupled to the understanding of the mechanisms that link the loss of *TSC1* (9q34) or *TSC2* (16p13.3) genes with the regulation of the Rheb/m-TOR/p70S6K pathway. These data have opened a new era in the comprehension of the pathogenesis of LAM and have also suggested new therapeutic strategies for this potentially lethal disease.**

Objective.—**To present and discuss the pathologic and molecular features of LAM within the spectrum of PEComas, providing a rational approach to their diagnosis.**

Data Sources.—**The published literature and personal experience.**

Conclusions.—**The inclusion of LAM within the PEComa category is supported by a variety of biologic data and can significantly help in providing a comprehensive view of this interesting and clinically relevant group of lesions. The demonstration of molecular alterations of the mTOR pathway in LAM and other PEComas represents a rational basis for innovative therapeutic approaches with inhibitors of mTOR signaling.**

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LYMPHANGIOLEIOMYOMATOSIS

Lymphangioleiomyomatosis (LAM) is a rare and progressive cystic lung disease (Figure 1) that affects mainly women during their reproductive years. Lymphangioleiomyomatosis is usually sporadic but patients with tuberous sclerosis complex (TSC)—an autosomal dominant genetic disease due to loss of either the *TSC1* (9q34) or *TSC2* (16p13.3) gene—are frequently affected. Accordingly, about one-third of patients with TSC suffer from LAM.[1–5] Lymphangioleiomyomatosis can be associated also with renal angiomyolipoma, a benign neoplasm composed of fat, vascular, and smooth muscle elements, both in sporadic and TSC-related cases.[6]

Intense investigation is focused on the search for new therapeutic options for LAM, since, in many cases, the progression to respiratory failure cannot be reversed and the only therapy is lung transplantation. In patients who do not undergo a transplant, the median survival is 8 to 10 years.[7] A better understanding of the pathogenesis of LAM is mandatory for defining new therapeutic options and huge efforts are devoted to this aim. A significant early improvement regarding LAM pathogenesis has been achieved by the recognition of LAM cells and their peculiar phenotype. Lymphangioleiomyomatosis consists of a nodular, often widespread and bilateral interstitial proliferation of smooth muscle cells, which can vary from small spindle-shaped cells to large epithelioid cells (Figures 2 and 3), usually arranged in a nodular and haphazard way around thin-walled, branching, lymphatic vascular channels (Figure 4); LAM is associated with dilated lymphatic vessels and cystic changes.[7] Lymphangioleiomyomatosis cells coexpress contractile proteins (mainly α–smooth muscle actin and desmin) (Figure 5), melanocytic markers such as HMB-45, HMSA-1, Melan-A/Mart1, and microphthalmia transcription factor (MiTF) (Figures 6 and 7) and estrogen and progesterone receptors.[8–16] The diagnosis of LAM can be difficult, particularly in small transbronchial biopsies, and in such cases immunohistochemical stains can be extremely useful.[9]

PERIVASCULAR EPITHELIOID CELLS AND PECOMAS

Early on, our group proposed LAM as a member of the ''perivascular epithelioid cell tumors,''[17–19] a wide family of proliferative lesions that we named PEComas and that has been widely recognized and incorporated in the World Health Organization nomenclature (PEComa: ''a mesenchymal tumor composed of histologically and immunohistochemically distinctive perivascular epithelioid cells'' [PECs]).[20] PEComas include angiomyolipoma (Figure 8), clear cell ''sugar'' tumor of the lung[21] and extrapulmonary sites,[22] clear cell myomelanocytic tumor

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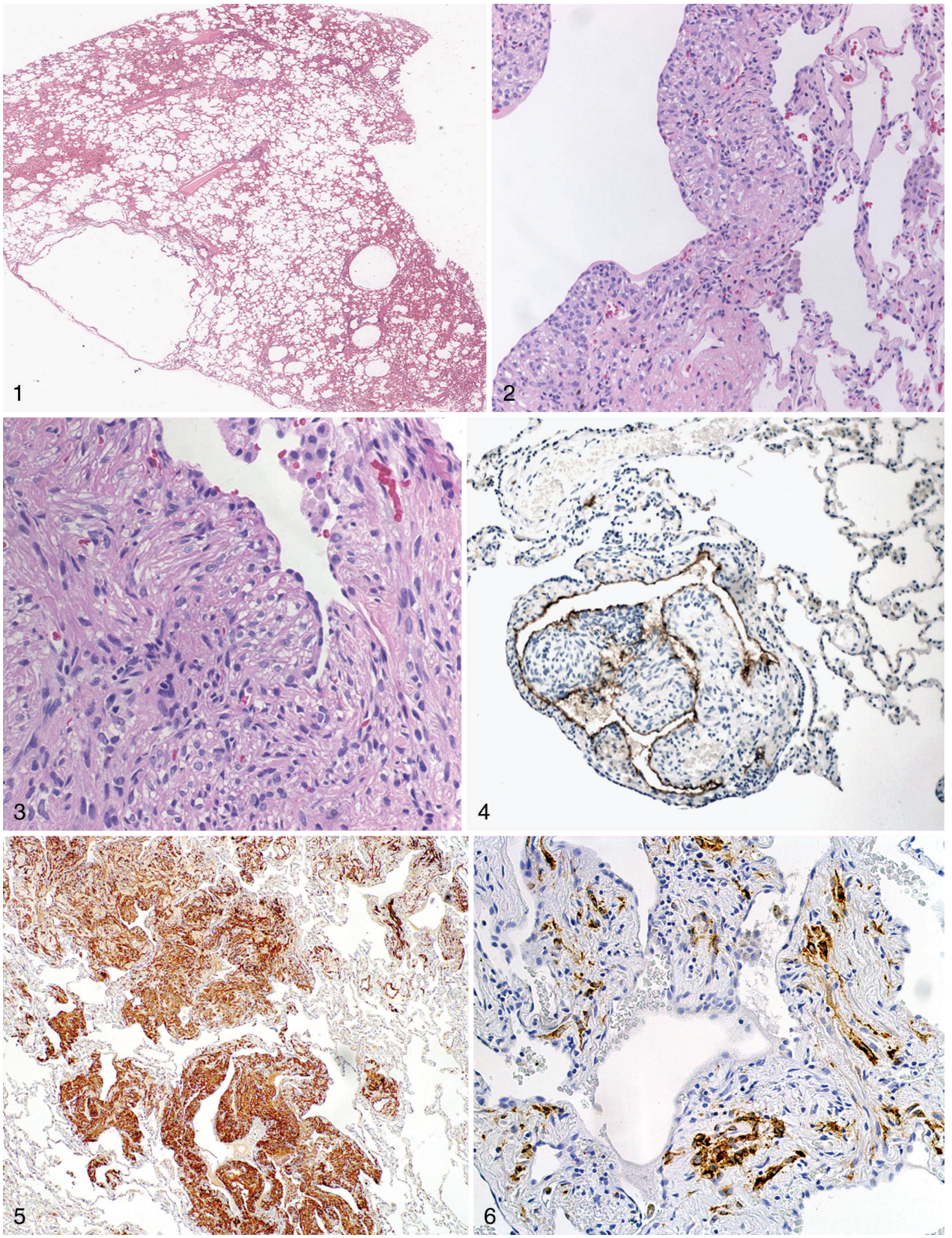

Figure 1. *Cystic appearance of pulmonary lymphangioleiomyomatosis (hematoxylin-eosin, original magnification ×2).*

Figure 2. *Pulmonary lymphangioleiomyomatosis: proliferation of spindle and epithelioid cells (hematoxylin-eosin, original magnification ×10).*

Figure 3. *Nodular proliferation prevalently composed of epithelioid cells in pulmonary lymphangioleiomyomatosis (hematoxylin-eosin, original magnification ×20).*

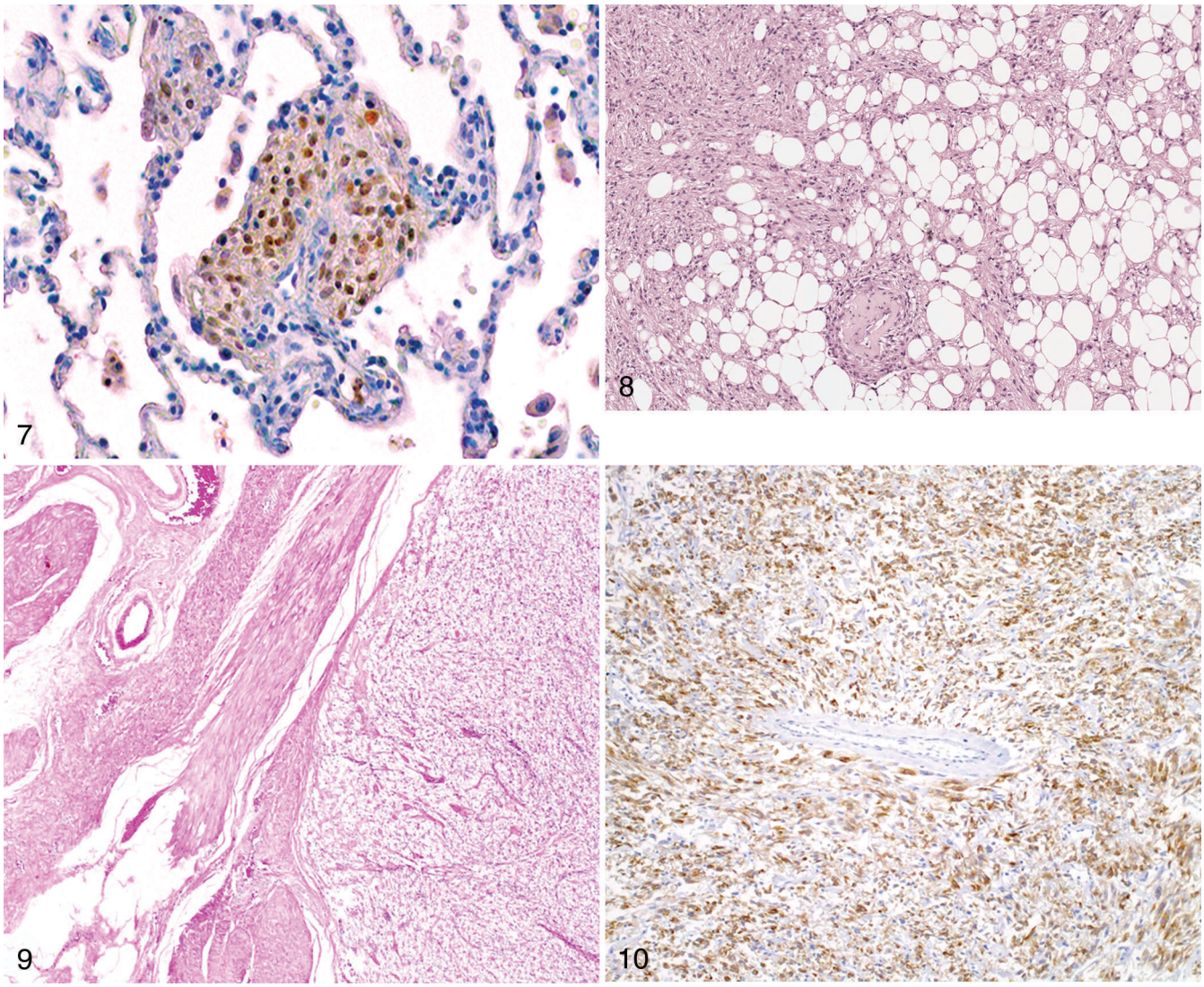

Figure 7. *Pulmonary lymphangioleiomyomatosis positive for MiTF immunostain (original magnification ×20).*

Figure 8. *Classic renal angiomyolipoma: the prototype of the proliferation of perivascular epithelioid cells (hematoxylin-eosin, original magnification ×10).*

Figure 9. *Clear cell myomelanocytic tumor of the bladder (hematoxylin-eosin, original magnification ×10).*

Figure 10. *Clear cell myomelanocytic tumor of the bladder positive for HMB-45 immunostain (original magnification ×20).*

of the falciform ligament/ligamentum teres,[23] and rare LAM-like tumors at other anatomic sites.[24] This heterogeneous group of neoplasms is unified by the presence of PECs, a mesenchymal cell type characterized by a distinct immunophenotypic profile with the coexpression of myogenic and melanocytic markers, such as HMB-45 (recognizing the gp100 protein), HMSA-1, Melan-A/Mart1, MiTF, α–smooth muscle actin, and, less commonly, desmin in different stages of modulation (Table).[17,24,25] Immunoreactivity for vimentin is usually inconspicuous in PECs. Morphologically, PECs are characterized by an epithelioid appearance with a clear to granular cytoplasm, a round to oval, centrally located nucleus, and an inconspicuous nucleolus.[17–19] Perivascular epithelioid cells also have ultrastructural distinctive features such as microfilament bundles with electron-dense condensation, numerous mitochondria, and membrane-bound dense granules.[9,26] In most PEC tumors, the cells, characterized by mild cytologic atypia, are observed at a perivascular location.[17–19] Although, at present, PECs do not have a

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Figure 4. *Podoplanin immunopositivity of endothelium highlights the arrangement of lymphangioleiomyomatosis cells around lymphatic vascular channels (original magnification ×15).*

Figure 5. *Pulmonary lymphangioleiomyomatosis positive for α–smooth muscle actin immunostain (original magnification ×4).*

Figure 6. *Pulmonary lymphangioleiomyomatosis positive for HMB-45 immunostain (original magnification ×20).*

Immunohistochemical Findings in Perivascular Epithelioid Cell Tumors										
	HMB-45	Melan-A	MiTF	ASMA	Desmin	Vimentin	S100	ER	PgR	CATH-K
LAM	+	+	+	+	+	+/−	−	+	+	+
Extrapulmonary LAM	+	not known	not known	+	+	+/−	−			not known
AML	+	+	+	+	+/−	+/−	−/+[a]	−/−/+	+/−	+
Epithelioid AML	+	+	+/−	−/+	−	−/+	−	−	−	not known
Clear cell ''sugar'' tumor of the lung	+	+	not known	−/+	−/+	−/+	−	−	−	not known
Clear cell ''sugar'' tumor of extrapulmonary sites	+	+/−	not known	−	−/+	−/+	−	−	−	not known
PEComa[b]	+	+/−	+	+/−	+/−	−/+	−	−	−	not known
Clear cell myomelanocytic tumor of the falciform ligament/ligamentum teres	+	+/−	+/−	+/−	−	not known	−	not known	not known	not known
Clear cell myomelanocytic tumor of other sites	+	+/−	not known	+	−	−/−/+	−	−	−	not known

Abbreviations: AML, angiomyolipoma; ASMA, α–smooth muscle actin; CATH-K, cathepsin K; ER, estrogen receptor; LAM, lymphangioleiomyomatosis; PEComa, perivascular epithelioid cell tumor; PgR, progesterone receptor.

[a] Adipocyte-like component.
[b] Uterus, urachal cyst, prostate, skin, etc.

known normal counterpart, it is possible to speculate that they are derived from genetically modified mesenchymal stem cells of perivascular origin.[27]

Although the inclusion of pulmonary LAM within this group of lesions has been questioned, LAM shares with PEComas most of their immunophenotypic features and typically shows the genetic alterations of the TSC, as we will discuss later.[1,16] The presence of lesions similar to LAM has been reported in extrapulmonary sites including mediastinal and retroperitoneal lymph nodes, soft tissue of the mesentery, and the renal sinus. Usually, extrapulmonary LAM presents as a localized, well-circumscribed mass called ''lymphangiomyoma.''[24]

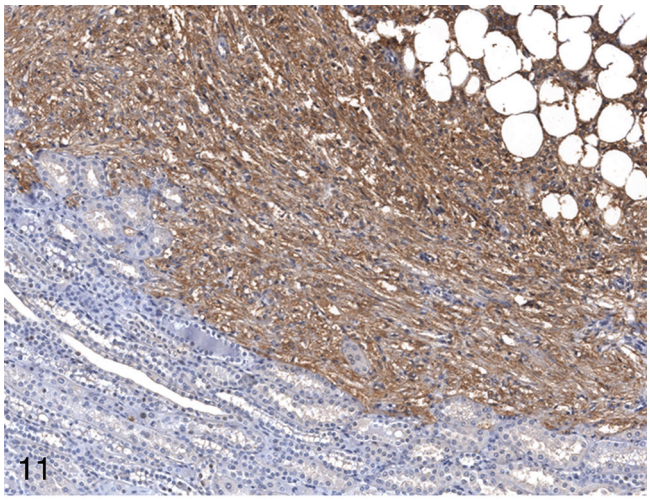
Depending on specific microenvironmental locations, PECs can modulate their morphology and immunophenotype. They can show either more pronounced muscle features and a spindle shape (stronger expression of α–smooth muscle actin and weaker expression of HMB-45), or they can exhibit more homogeneous epithelioid morphology with strong positivity for HMB-45 and a mild expression, if any, for α–smooth muscle actin. Perivascular epithelioid cells can also acquire adipocytic features with large vacuolated cytoplasms.

The adipocyte morphology is consistently represented in PECs forming angiomyolipomas (AMLs) in which a variable mixture of adipose tissue and spindle and epithelioid smooth muscle cells, together with abnormal thick-walled blood vessels, are typical; these features represent the prototype for the ability of PECs to modulate their appearance (Figure 8).[28–31] Most frequently, AMLs occur in the kidney and comprise a variety of forms including classic angiomyolipoma, microscopic angiomyolipoma (so-called microhamartoma), intraglomerular lesion, cystic angiomyolipoma, epithelioid angiomyolipoma, and oncocytoma-like angiomyolipoma. Angiomyolipomas can also occur in other anatomic sites, as previously described.[18,30,32–34] Classic AMLs are the most common mesenchymal tumors of the kidney; this tumor can be sporadic (in 80% of cases) or associated with TSC (in 20% of cases). Sporadic AMLs, single and unilateral, occur in the fourth to sixth decade of life, with a female predominance. For patients with TSC, however, AMLs occur at a younger age (the third and fourth decade of life) in both sexes and are usually asymptomatic, bilateral, smaller than sporadic AMLs, and multifocal.[28] In addition to AMLs, other types of PEComas can develop in kidney, such as lymphangioleiomyomatosis of the renal sinus. Apart from those affecting lungs and kidneys, PEComas have been described in a variety of anatomic sites such as the uterus,[19,35] bladder,[36] prostate,[37] and several other organs (Figures 9 and 10).[38,39]

MOLECULAR PATHOGENESIS OF LYMPHANGIOLEIOMYOMATOSIS AND PECOMAS

The concept of PEComas as a distinctive tumor entity has been strongly strengthened by the demonstration of common and distinctive molecular alterations in these neoplasms, no matter where they are found and how they are named (AML, LAM, or something else). The development of PEComas is in fact related to inactivating losses of gene *TSC1* (9q34) or *TSC2* (16p13.3).[1,5] *TSC1* and *TSC2* encode hamartin and tuberin, respectively—2 cytosolic proteins that interact strictly and function together as a heterodimer[40] and participate in signaling pathways that control cell growth and proliferation. In particular, TSC genes (*TSC1* and *TSC2*) have an important role in the regulation of cell cycle via the Rheb/mTOR/p70S6K pathway.[41] Accordingly, tuberin has a Rheb GTPase-activating protein activity which inactivates Rheb; alterations of tuberin lead to hyperactivation of Rheb, with subsequent stimulation of the mTOR pathway.[6] Mammalian target of rapamycin (mTOR) is a family member of phosphatidylinositol 3-kinase (PI3-kinase)–related kinases, which controls the activity of the translational inducer S6K1 (S6 kinase 1 or p70 S6 kinase 1), and the translational inhibitor 4E-BP1 (eIF4E binding protein 1). The activation of mTOR leads to cell growth and proliferation.[42]

A fundamental step in the understanding of the pathogenesis of PEComas was the demonstration, more than 10 years ago, of the loss of heterozigosity (LOH) of chromosome arm 16p (containing the *TSC2* locus) in both



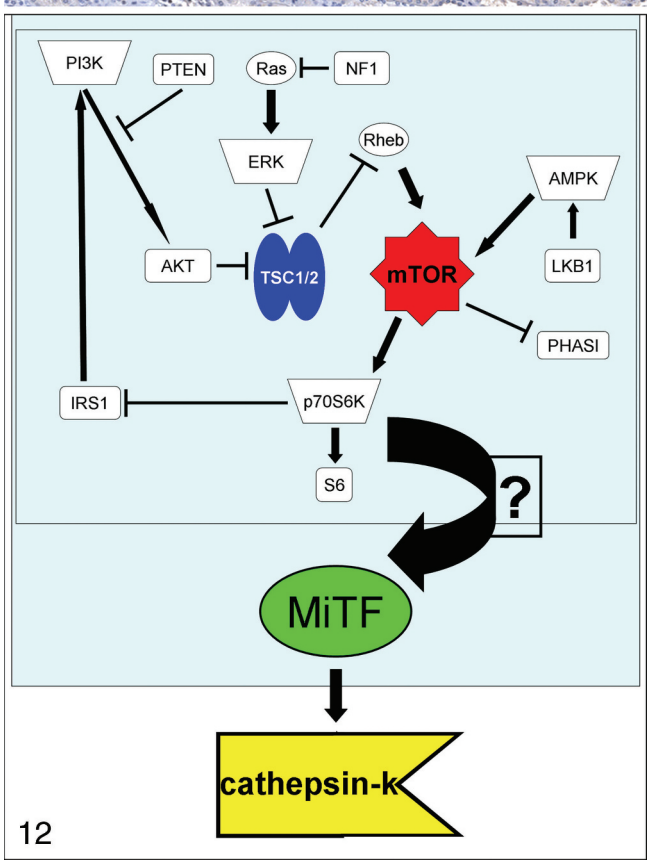


Figure 11. *Renal angiomyolipoma: cytoplasmic signal in immunohistochemical reaction for p70S6K (original magnification ×10).*

Figure 12. *Hypothesis of a link between mTOR pathway and the microphthalmia transcription factor network. Abbreviations: AMPK, AMP-activated protein kinase; ERK, extracellular signal–regulated kinase; IRS1, insulin receptor substrate 1; MiTF, microphthalmia transcription factor; mTOR, mammalian target of rapamycin; NF1, neurofibromin 1; PHASI, phosphorylated heat- and acid-stable I; PI3K, phosphoinositide 3–kinase; PTEN, phosphatase and tensin homolog; p70S6K, p70S6 kinase; Rheb, Ras homolog enriched in brain; TSC1/2, tuberous sclerosis complex gene 1 and 2.*

the inherited and sporadic forms of AML, with occasional LOH of the *TSC1* gene.[43] Recurrent chromosomal alterations have been demonstrated in sporadic cases in renal and extrarenal PEComas, both occurring within the TSC.[44] In particular, deletions occurring on chromosome arm 16p have been demonstrated in 6 PEComas at different anatomic sites (3 renal PEComas; 1 prostatic, 1 uterine, and 1 pelvic PEComa). In addition, LOH involving the *TSC2* locus has been shown in 12 other PEComas (5 classic AMLs and 7 PEComas at different anatomic sites). Loss of heterozigosity in both the *TSC2* and the *TSC1* locus was reported in 1 single renal PEComa in a patient with TSC.[45]

Similar abnormalities affecting the *TSC2* locus have been described in both sporadic and TSC-associated LAM cases.[6,46–50] Increased levels of phospho-p70S6K, a reliable marker of mTOR activity, has been recently demonstrated in sporadic renal and extrarenal AML, with associated reduction of the phospho-AKT expression (Figure 11).[51,52] These findings are consistent with the disruption of *TSC1/2* function. In line with these observations is the clinical amelioration for both AML and LAM—with regression of tumor size and performance improvement—by sirolimus, an immunosuppressive agent that suppresses mTOR signaling.[53–55]

LINK BETWEEN THE EXPRESSION OF MELANOCYTE-SPECIFIC PROTEINS OBSERVED IN LYMPHANGIOLEIOMYOMATOSIS AND PECOMA CELLS AND THEIR MOLECULAR ALTERATIONS IN *TSC2* GENE AND M-TOR PATHWAY

To summarize what we know to date, PEComas are neoplasms exhibiting a peculiar protein expression profile (HMB-45, MiTF, Melan-A/Mart1, α–smooth muscle actin expression) and all are characterized by specific molecular alterations related to the TSC (LOH in *TSC2* and mTOR pathway activation). Nevertheless, it is currently unknown whether such molecular changes are related to such a peculiar protein expression pattern. Is there any link between the expression of melanocyte-specific proteins observed in LAM and PEComa cells and the molecular alterations in *TSC2* gene and mTOR pathway? Microphthalmia transcription factor is a member of the β–helix-loop-helix leucine zipper (β-HLH-Zip) family of transcription factors. This transcription factor plays a relevant role in the differentiation and/or the functional features of several cell types including osteoclasts, melanocytes, mast cells, and natural killer cells.[56–58] How this factor can exert these different functions in the tissue-regulated expression of different proteins in multiple cell types is currently incompletely understood, but it is likely related to the variety of isoforms of MiTF, each differing in its promoter and initial exon usage.[59]

Looking for a common link between melanogenesis, MiTF, and the mTOR pathway, we focused our attention on the expression pattern of cathepsin K. A papain-like cysteine proteinase with high matrix-degrading activity, cathepsin K is under the transcriptional control of MiTF; it is expressed in melanoma cells and typically at high levels in osteoclasts, where it is crucial for bone resorption.[60–65] Interestingly, mTOR pathway is also involved in the stimulation of cell survival in osteoclasts and melanoma cells,[66–68] and everolimus, a derivative of rapamycin, which targets the mTOR pathway, is able to specifically suppress cathepsin K expression in osteoclasts.[69] By combining all these data, it is possible to speculate that both the transcription factor MiTF (which belongs to the protein expression profile of PEComas) and mTOR (whose pathway is altered in PEComas, through the *TSC2* mutations and after tuberin dysfunction) may be

related to the expression of cathepsin K in PEComas (Figure 12). In a recent study, Chilosi et al[70] investigated this issue by analyzing the expression of cathepsin K in a large series of PEComas and demonstrated that, in fact, both AML and LAM strongly expressed this protease. In our view, cathepsin K represents a reliable diagnostic marker for LAM cells and can also explain some features of the disease, such as the typical formation of lung cysts, by its powerful matrix-degrading activity on collagen and elastin. Finally, it is possible to speculate that mTOR inhibitors, recently proposed as a new therapeutic option for LAM and other PEComas, may work, in part, by limiting the activity of cathepsin K, thus contrasting with the destructive remodelling of lung structure.

LYMPHANGIOLEIOMYOMATOSIS CELLS AS NEOPLASTIC CELLS

Although the neoplastic, benign nature of angiomyolipoma can be defined by its tumoral growth within the renal parenchyma, the definition of LAM as a neoplastic disease is far less obvious, and only its inclusion within the PEComa family—with demonstration of identical molecular abnormalities—has provided the elements for such a definition. As a consequence of the abrogation of the regulatory functions of TSC gene products, due to inactivating mutations of either *TSC1* or *TSC2*, the mTOR pathway is abnormally activated in LAM and other PEComas; this provides the abnormal cells with a significant survival advantage. In fact, tuberin can negatively regulate the activity of protein S6 and p70S6K, specifically, and disruption of this mechanism can explain the abnormal cell growth in PEComas.[71] Accordingly, although LAM cells do not exhibit atypia or elevated proliferation, the progressive infiltration of lung parenchyma can be considered as evidence of a metastatic spread, and LAM can accordingly recur after lung transplantation.[72] In PEComas, the survival advantage provided by the genetic defect in TSC is likely limited, since most of the developing tumors are benign. In fact, the genetic abnormalities characterizing LAM and PEComas affect tumor suppressor genes, leading to cell clones that maintain their dependency on availability of growth factors such as epidermal growth factor.[73] A number of malignant neoplasms have been described that share the phenotype and the genetic abnormalities characterizing PEComas,[74–76] but malignant transformation in PEComas most likely needs further genetic modifications.[77]

Great interest has focused on the possible hormonal dependence of LAM progression because of the striking gender distribution occurring in LAM, the reported disease progression during pregnancy or following exogenous estrogen administration, as well as the variable expression of steroid hormone receptors by LAM cells.[12,29,78–81] All these observations suggest the involvement of estrogens in the pathogenesis of LAM.[79] The possible therapeutic significance of hormonal manipulation has been investigated, but with discordant results.[81–84] Recent evidence has shown a direct role of estrogens in promoting survival of tuberin-null cells, as well as in the invasion and destruction of lung parenchyma, induced by overproduction of matrix metalloproteinases.[85,86] Expression of CD44v6, a marker involved in homing during metastasis, is in line with this model.[86]

All this evidence suggests a model in which LAM cells are able to infiltrate the lung through a metastatic dissemination.[47,72,86,88] Further studies are needed to determine the source of metastasizing PEC cells in pulmonary LAM , that is, to determine whether they derive from neoplastic aggregates (eg, angiomyolipomas) or from mesenchymal precursors homing in microenvironmental perivascular niches.

CONCLUSION

Tremendous improvement has been made in the last few years concerning the molecular pathology and the genetic features of LAM and other PEComas, which opens the way to new therapeutic options. Nevertheless, a number of relevant biologic issues regarding this interesting class of tumors are still open. It is not clear why only a limited number of tumor types (AML, LAM, and other PEComas) develop in patients with tuberous sclerosis, since the genetic defect could affect the lifespan of all cell types. It is possible that the defect acts only on a subset of susceptible cell types (eg, perivascular mesenchymal stem cells) and that, upon mutation of the mTOR pathway, these abnormal cells acquire an abnormal phenotype and behavior but still maintain a limited differentiation potential, with eventual accumulation within the perivascular microenvironment. Accordingly, in LAM, a close relation with lymphatic channels has been demonstrated.[89] Further studies are needed to better define the nature and properties of the PEC cell precursors and, if they exist, their normal counterparts.

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