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Therapeutic options for lymphangioleiomyomatosis (LAM): where we are and where we are going

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Abstract

Lymphangioleiomyomatosis (LAM), a multisystem disease affecting predominantly premenopausal and middle-aged women, causes progressive respiratory failure due to cystic lung destruction and is associated with lymphatic and kidney tumors. In the past, the treatment of LAM comprised exclusively anti-estrogen and related hormonal therapies. These treatments, however, have not been proven effective. In this article, we discuss new findings regarding the molecular mechanisms involved in the regulation of LAM cell growth, which may offer opportunities to develop effective and targeted therapeutic agents.

Introduction and context

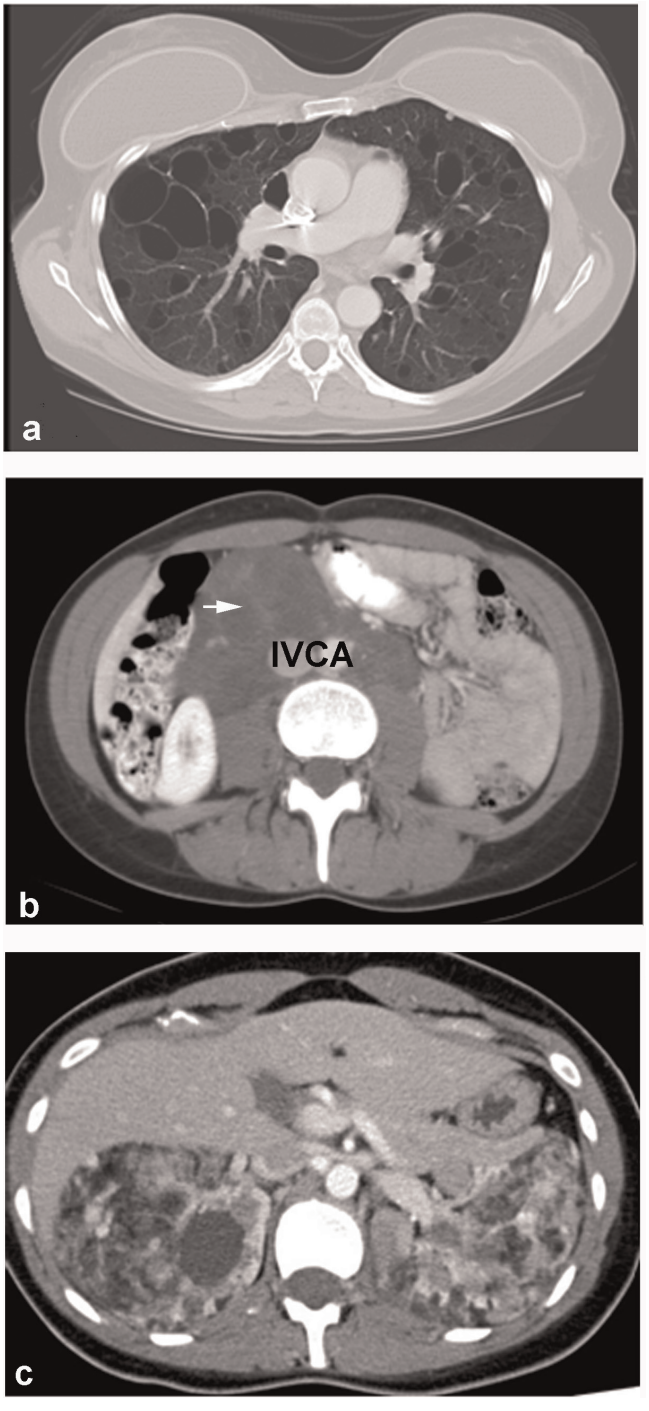
Lymphangioleiomyomatosis (LAM) is a multisystem disease affecting predominantly premenopausal and middle-aged women and is characterized by proliferation of abnormal smooth muscle-like cells (LAM cells) [1]. LAM is associated with cystic lung destruction, fluid-filled cystic tumors in the axial lymphatics (for example, lymphangioleiomyomas), and abdominal tumors (for example, angiomyolipomas), primarily in the kidneys, comprising adipocytes, vascular structures, and smooth muscle cells [1]. LAM occurs in about one-third of women with tuberous sclerosis complex (TSC), an autosomal dominant disorder, with variable penetrance. TSC occurs in 1 of 5800 live births [2], results from mutations in the *TSC1* or *TSC2* genes [2], and is characterized by hamartoma-like tumor growths in various organs, cerebral calcifications, seizures, and mental retardation. Sporadic LAM is a relatively uncommon disease, with a prevalence that has been estimated at 1-2.6 per million women [3].

The tumor suppressor genes, *TSC1* and *TSC2*, have been implicated in the etiology of sporadic LAM because mutations and loss of heterozygosity in the *TSC* genes have been detected in LAM cells [4,5]. *TSC1* encodes hamartin, a protein that plays a role in the reorganization of the actin cytoskeleton, and *TSC2* encodes tuberin, a protein with roles in cell growth and proliferation [4,5].

LAM presents with dyspnea, pneumothorax, chylothorax, ascites, or angiomyolipoma-derived abdominal hemorrhage [1]. Imaging studies show numerous thin-walled cysts throughout the lungs, angiomyolipomas, and lymphangioleiomyomas (Figure 1). Pulmonary function tests show reduced expiratory flow rates or lung diffusion capacity or both [1].

Because LAM is predominantly a disease of premenopausal women and may worsen during pregnancy [6] or following the administration of estrogens [7], hormonal manipulations have been employed in its treatment. However, no controlled studies have been undertaken to determine their efficacy. In a retrospective study, we found no difference in disease progression between patients treated with or without progesterone [8]. Suppression of ovarian function, either by oophorectomy or gonadotropin-releasing hormone (GnRH) analogs, also did not appear to benefit patients [8,9]. A trend

Figure 1. Computed tomography scans of patients with lymphangioleiomyomatosis



(a) Numerous thin-walled cysts distributed throughout the lungs. **(b)** A large lymphangioleiomyoma (arrow) located in the retroperitoneal area surrounding the aorta (A) and inferior vena cava (IVC). **(c)** Angiomyolipomas involving both kidneys.

toward decreased rates of functional decline in postmenopausal patients has been noted [8]. Overall, at present, treatment of LAM involves supportive care, management of complications (for example, pneumothorax, pleural effusions, and ascites), bronchodilators (as needed for asthma-like symptoms), oxygen therapy, and (in cases of respiratory failure) lung transplantation.

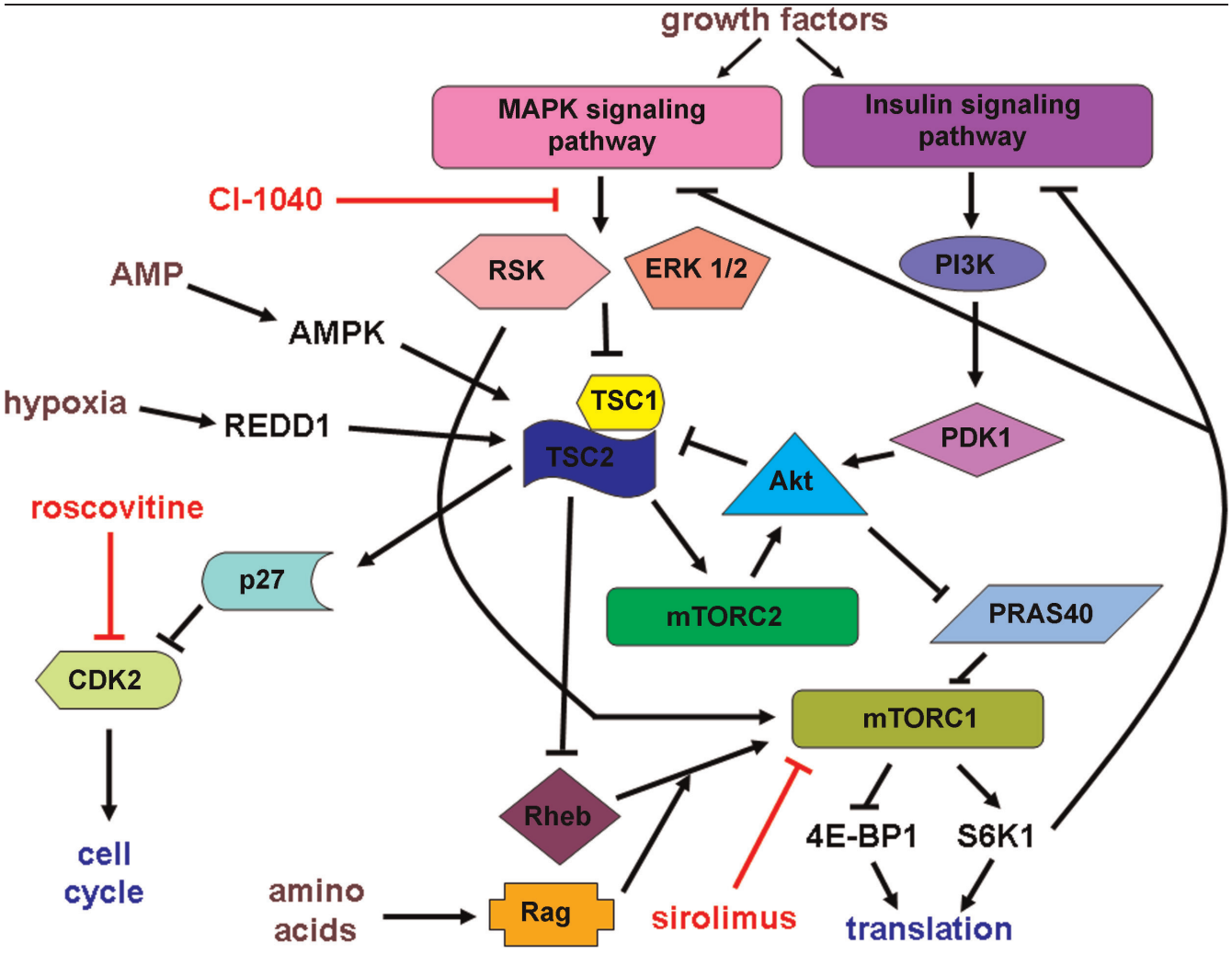
Recent advances

Inhibitors of mammalian target of rapamycin

TSC1 and *TSC2* are tumor suppressor genes that encode hamartin and tuberin, respectively [10,11]. Hamartin and tuberin may have individual functions, but they also interact to form a cytosolic complex. Hamartin functions in the reorganization of the actin cytoskeleton by interacting with the ezrin-radixin-moesin family of proteins [12]. Tuberin has roles in pathways controlling cell growth and proliferation (Figure 2) [13]. It is described as a negative regulator of cell cycle progression since the loss of tuberin shortens the G_1 phase of the cell cycle. Tuberin binds p27Kip1, a cyclin-dependent kinase (CDK) inhibitor, preventing its degradation and leading to inhibition of the cell cycle. In the absence of tuberin, p27 becomes mislocalized in the cytoplasm, allowing the cell cycle to progress [13].

The TSC1/2 complex acts upstream of the intracellular serine/threonine kinase mammalian target of rapamycin (mTOR) and mediates growth factor, energy, and stress signals, thereby regulating cell growth and proliferation. There are two different complexes that contain mTOR: mTORC1, which contains raptor (regulatory associated protein of mTOR), and mTORC2, which contains rictor (rapamycin-insensitive companion of mTOR) [14-16]. TSC1/2 positively regulates mTORC2, leading to phosphorylation and activation of protein kinase B (Akt) [17,18], while it negatively regulates mTORC1. In the presence of growth factors, both the mitogen-activated protein kinase (MAPK) and insulin signaling pathways can be activated, resulting in inhibition of TSC1/2 through phosphorylation of TSC2 by p90 ribosomal S6 kinase (RSK), extracellular signal-regulated kinase (ERK1/2), or Akt [19-22]. TSC2 acts as a GTPase-activating protein (GAP) for Ras homolog enriched in brain (Rheb), promoting the formation of inactive Rheb-GDP from the active Rheb-GTP [23-25]. Inhibition of TSC1/2 by growth factor stimulation inhibits the GAP activity and allows accumulation of active Rheb-GTP. Rheb-GTP stimulates mTORC1, which phosphorylates substrates such as ribosomal S6 kinases and eukaryotic

Figure 2. TSC1/2 integrates multiple signals to control cell growth and proliferation



Growth factors stimulate the MAPK and insulin signaling pathways, leading to TSC2 phosphorylation and inactivation. TSC1/2 negatively regulates mTORC1 through its actions on Rheb, while it positively regulates mTORC2. The insulin signaling pathway can activate mTORC1 without going through TSC1/2 by Akt phosphorylation of PRAS40, an inhibitor of mTORC1, thereby relieving the inhibition. Similarly, the MAPK signaling pathway can activate mTORC1 without going through TSC1/2 via RSK phosphorylation of raptor, a component of the mTORC1 complex, leading to mTORC1 activation. Activation of mTORC1 leads to protein translation and to a negative feedback loop on the activation of the insulin and MAPK signaling pathways. In the presence of amino acids, the Rag GTPase heterodimers promote the localization of mTORC1 to cellular compartments containing Rheb, thereby promoting mTORC1 activation. In conditions of low cellular energy or hypoxia, AMPK phosphorylates and activates TSC2, while hypoxia increases the transcription of REDD1, which also activates TSC2, leading to inhibition of translation. TSC2 binds p27Kip1, a cyclin-dependent kinase inhibitor, stabilizing it and resulting in inhibition of cell cycle progression. Sirolimus, or rapamycin, inhibits mTORC1, while CI-1040 is an MAPK/ERK inhibitor. Roscovitine is an inhibitor of CDK2. 4E-BP1, factor 4E-binding protein 1; Akt, protein kinase B; AMPK, AMP-dependent protein kinase; CDK2, cyclin-dependent kinase 2; ERK1/2, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; mTORC, mammalian target of rapamycin complex; PDK1, pyruvate dehydrogenase kinase, isozyme 1; PI3K, phosphoinositide 3-kinase; PRAS40, proline-rich protein kinase B substrate of 40 kDa; Rag, Ras-related small GTP-binding protein; REDD1, regulated in the development and DNA damage response 1; Rheb, Ras homolog enriched in brain; RSK, p90 ribosomal S6 kinase; S6K1, S6 kinase 1; TSC, tuberous sclerosis complex.

initiation factor 4E-binding proteins, leading to enhanced protein translation [26].

Both the MAPK and insulin signaling pathways can affect mTORC1 without involving TSC1/2. RSK can phosphorylate raptor of mTORC1 [27], thus promoting mTORC1 kinase activity directly. Akt phosphorylates PRAS40 (proline-rich Akt substrate of 40 kDa), an inhibitor of mTORC1, and relieves this inhibition [28].

TSC1/2 also integrates signals indicating amino acid levels and energy status, both of which are necessary to

fuel translation. Under conditions of high intracellular levels of AMP (indicating energy stress), AMP-dependent protein kinase (AMPK) phosphorylates and activates TSC2, thereby inhibiting mTORC1 and translation [29]. Hypoxia can inhibit mTORC1 activity both by stimulation of AMPK (as oxygen is necessary for the production of ATP by oxidative phosphorylation) and by increasing the transcription of REDD1 (regulated in development and DNA damage response 1), which activates TSC2 [30]. Amino acids promote GTP binding by Rag (Ras-related small GTP-binding protein)-GTPase heterodimers, which bind raptor, promoting mTORC1 localization in cellular compartments where Rheb is present. This allows mTORC1 activation only when there are sufficient amino acids to support translation [31].

Rapamycin, or sirolimus, forms a complex with FKBP-12 (FK506-binding protein-12) that binds and inhibits mTORC1 [32]. mTORC1 is acutely inhibited by rapamycin, whereas mTORC2 responds only to prolonged rapamycin treatment and concentration [33-36]. Sirolimus decreased tumor size in a rat model of TSC with a functionally null germline mutation of *Tsc2*, which spontaneously develops renal cell carcinomas [37]. There were, however, rare persistent renal tumors, which suggest some resistance to rapamycin. In patients with angiomyolipomas, tumor size decreased by half after 1 year of sirolimus therapy, while lung function was improved in some patients [38]. Without sirolimus, however, the angiolipomas regained size. A second report suggests that sirolimus may inhibit the decline in lung function rather than improve function [39]. A Multicenter International Lymphangioleiomyomatosis Efficacy of Sirolimus Trial (University of Cincinnati Medical Center, Cincinnati, OH, USA; Frank McCormack, principal investigator) to examine the effect of rapamycin on pulmonary function is in progress.

It has been found that, due to upregulation of receptor tyrosine kinases, inhibition of mTORC1 results in the activation of Akt [40-42]. Due to activation of the S6K PI3K (phosphoinositide 3-kinase)-Ras pathway, mTORC1 inhibition also leads to the activation of the ERK/MAPK cascade [43]. The activation of the Akt and ERK/MAPK signaling pathways may partially explain why rapamycin treatment has not been found to be completely successful. Inhibition of the MAPK pathway along with use of rapamycin has been found to be more efficient at blocking mouse $Tsc2^{-/-}$ cell proliferation than either inhibitor alone [44].

Estrogens

Several observations implicate estrogens in the pathogenesis of LAM. Estradiol stimulated growth of human angiomyolipoma $TSC2^{-/-}$ cells [45] and pulmonary metastasis of $Tsc2^{-/-}$ Eker rat uterine leiomyoma-derived smooth muscle (ELT3) cells in mice [46], which was associated with activation of p42/44 MAPK. Estrogen enhanced the survival and colonization of intravenously injected $Tsc\ 2^{-/-}$ cells in mice [46], an effect prevented by the MAPK/ERK kinase (MEK) inhibitor CI-1040 [46]. Estrogen receptor activation also increased matrix metalloproteinase (MMP)-2 activity of LAM cells, promoting LAM cell invasiveness, and doxycycline, an antibiotic with anti-MMP activity, inhibited this effect, suggesting an estrogen-MMP-driven process in lung destruction and LAM cell metastasis [47]. Finally, estrogens accelerated growth of angiomyolipoma cells in a xenograft tumor system [48]. These data suggest that blockade of the MEK pathway and inhibition of MMP production could be new potential approaches to the treatment of LAM. Furthermore, anti-estrogen therapy may have a role in LAM, but timing it to preclude LAM cell metastasis and survival may be crucial.

Matrix metaloproteinases

Since MMPs are present within LAM lesions [49,50], doxycycline (an MMP inhibitor that affects growth and migration of neoplastic cells, angiogenesis, lymphangiogenesis, and smooth muscle cell growth [51,52]) could also be a therapeutic alternative. Recently, it was reported that doxycycline therapy decreased urinary MMP levels and improved lung function in a patient with LAM [53]. A double-blind placebo-controlled clinical trial is planned at the University of Nottingham, UK (Simon Johnson, principal investigator) to evaluate the effects of doxycycline in LAM.

Statins

Statins are 3-hydroxy-3-methyl-glutaryl-coenzyme-A reductase inhibitors that may inhibit both rapamycin-sensitive and rapamycin-insensitive mechanisms of tuberin-null cell growth [54]. Indeed, statins block the growth of $Tsc2^{-/-}$ mouse embryo fibroblasts and ELT3 smooth muscle cells [54]. Statins did not, however, decrease cystadenoma size in $Tsc2^{+/-}$ mice [55]. Furthermore, therapeutic success with statins was not observed in TSC, and no correlation between statin use and angiomyolipoma response to sirolimus in patients with TSC or sporadic LAM was observed [18]. In a retrospective study, the rate of decline in lung diffusion for patients on statins was greater than that of their matched controls [56].

Interferon, vascular endothelium growth factors, and cyclin-dependent kinase 2 inhibitors

The combination of rapamycin and interferon (IFN)-γ was not more effective than rapamycin alone against

TSC-related kidney tumors in *Tsc2*$^{+/-}$ mice [57]. IFN-β, which is expressed in LAM tissues and LAM cell cultures, attenuated proliferation of LAM-derived and *Tsc2*-null ELT3 cells, but this effect was potentiated by rapamycin [58]. In a subcutaneous *Tsc2*$^{-/-}$ tumor mouse model, sorafenib, a vascular endothelial growth factor (VEGF) receptor inhibitor, and sirolimus together increased survival and decreased tumor volume more effectively than sirolimus alone [57].

Other potential therapeutic options for LAM are CDK2 inhibitors [59,60]. Tuberin negatively regulates the activity of CDK2, binding to the CDK inhibitor p27 (a major regulator of cell cycle progression), preventing its degradation, and thereby increasing the amount of p27 bound to CDK2. In tuberin-negative cells, p27 is degraded and delocalized to the cytoplasm [59,60]. This results in lack of inhibition of CDK activities in the cell nucleus by p27. Therefore, inhibitors of CDK2, such as roscovitine, a new potential anti-cancer agent [61], could perhaps have a role in the therapy of LAM.

Pneumothorax

The highest incidence of pneumothorax among patients with chronic lung diseases occurs in LAM, with multiple recurrent pneumothorax occurring in 70% of patients [1,62]. Generally, cyst size parallels the incidence of pneumothorax; a higher incidence of pneumothorax is seen in patients with larger cysts [62,63]. Furthermore, pneumothoraces are associated with faster decline in lung function in patients with mild disease [62,63].

In general, small pneumothoraces can be treated conservatively or, if they do not resolve, by closed thoracostomy. If air leak persists, the lung does not expand, or the pneumothorax recurs, chemical or surgical pleurodesis by video-assisted thoracoscopy is recommended [64]. Because of the high rate of pneumothorax recurrence in LAM, pleurodesis at the time of initial pneumothorax occurrence is recommended [64]. Talc pleurodesis is the most effective but may result in unwanted fibrothorax that can complicate removal of the lung at the time of transplantation [64].

Lymphangioleiomyomas, chylothorax, and ascites

Lymphatic involvement in LAM occurs in the posterior mediastinum and in retroperitoneal and pelvic areas and includes lymphadenopathy and lymphangioleiomyomas [65]. Thoracic and abdomino-pelvic lymphangioleiomyomas are observed in 16-21% of patients with LAM. On computed tomography scans, tumors appear as well-circumscribed masses of variable dimensions, comprising a wall and a central fluid-rich region (Figure 1b) [65]. These tumor masses are probably caused by the proliferation of LAM cells, leading to compression or obstruction of lymphatic vessels and chylous effusions. Of importance in differentiating lymphangioleiomyomas from abdominal malignancies is the fact that lymphangioleiomyomas exhibit a diurnal variation in size [66,67].

Serum VEGF-D, a lymphangiogenic factor, is increased in the serum of patients with LAM compared with normal individuals and appears to be a measure of lymphatic involvement in LAM [68-70]. Lymphangioleiomyomas may cause pain, neuropathy, abdominal bloating, urinary frequency, and edema, suggesting a lymphoproliferative disease [65,71-73]. Surgical resection of lymphangioleiomyomas is not recommended for standard care as it may lead to lymphatic leakage, chylothorax, and ascites.

Chylous effusions, including pleural effusions, are particularly difficult to treat [74,75]. Low-fat diet with medium-chain triglycerides and therapeutic thoracentesis should be attempted initially, however, most patients require pleurodesis [75]. After pleurodesis, a low-fat diet with medium-chain triglycerides is recommended.

A pleuro-peritoneal or peritoneal-venous shunt may be considered for the treatment of chylothorax or ascites when the effusions are disabling and cause mechanical/nutritional problems, but little experience with these therapeutic modalities in LAM has been reported [76,77]. Treatment with somatostatin and octreotide may be considered for those patients with recurrent pleural effusions or disabling ascites. Treatment with these agents produced a successful reduction in chylous effusions, chyluria, ascites, and peripheral lymphedema in other clinical settings, such as idiopathic congenital chylothorax, lymphoma, traumatic lymphatic injury, and yellow nail syndrome [78-80]. Anti-VEGF-D therapies may eventually become the best therapeutic option for the treatment of these lymphatic disorders.

Angiomyolipomas

Angiomyolipomas of less than 4 cm in diameter are well tolerated and are usually associated with well-preserved renal function (Figure 1c). The principal complication of larger angiomyolipomas is bleeding, which may cause flank pain and bloody urine [81-83]. In this setting, embolization of the tumor, rather than surgery, is recommended to preserve kidney function. Intractable pain is also an indication for selective embolization of the tumor [81,82]. Prophylactic embolization can be undertaken in patients who have large angiomyolipomas

and no known episodes of bleeding, but evidence favoring this approach is still lacking [83]. Not infrequently, the blood supply of these tumors is complex, comprising abnormal aberrant vasculature that may prevent successful and safe arterial embolization. If surgical therapy is being considered, all efforts should be made to preserve the kidney [83].

Implications for clinical practice

In the last 10 years, there has been great progress in understanding the natural history of LAM, its pathogenesis, and the biology of the LAM cell. Progress in therapy, however, has come slowly. Despite a probable role of estrogens in the pathogenesis of LAM, there is currently no evidence that suppression of estrogen secretion by oophorectomy or GnRH analogs or treatment with progesterone is effective in LAM. New treatments based on inhibition of the MAPK/ERK kinase pathway may be proven to be effective. At present, inhibitors of regulators of cell proliferation, specifically mTOR inhibitors such as sirolimus or everolimus, appear to be the most promising therapeutic agents, although significant toxicity associated with long-term therapy could be a major problem. As a role of lymphangiogenesis and VEGF-D in the pathogenesis of LAM is recognized, it may be possible (as in the case of vascular tumors and malformations) to develop effective anti-lymphangionesis agents in LAM. Although a number of drug trials are under way to test some of these therapies (in particular, inhibition of mTOR), more research to discover new treatments is warranted. As in cancer, the combination of several agents may offer the best hope for effective therapy.

Abbreviations

Akt, protein kinase B; AMPK, AMP-dependent protein kinase; CDK, cyclin-dependent kinase; ELT3, Eker rat uterine leiomyoma-derived smooth muscle; ERK, extracellular signal-regulated kinase; GAP, GTPase-activating protein; GnRH, gonadotropin-releasing hormone; IFN, interferon; LAM, lymphangioleiomyomatosis; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; mTORC, mammalian target of rapamycin complex; raptor, regulatory associated protein of mTOR; Rheb, Ras homolog enriched in brain; RSK, p90 ribosomal S6 kinase; TSC, tuberous sclerosis complex; VEGF, vascular endothelial growth factor.

Competing interests

The authors declare that they have no competing interests.

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