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

Angelo M Taveira-DaSilva*, Wendy K Steagall and Joel Moss

Address: Translational Medicine Branch, Building 10, Room 6D05, MSC 1590, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892-1590, USA

* Corresponding author: Angelo M Taveira-DaSilva (dasilvaa@nhlbi.nih.gov)

F1000 Medicine Reports 2009, 1:93 (doi:10.3410/M1-93)

The electronic version of this article is the complete one and can be found at: http://F1000.com/Reports/Medicine/content/1/93

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...
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 G1 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 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.
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 Rap (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 angioliomas 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 evaluate the effects 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/MEK 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 Tsc2−/− 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 metalloproteinases**

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 tuherin-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 chyloous 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.

Chyloous 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 chyloous 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-lymphangiogenesis 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, regulator 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.

Acknowledgments
The authors thank Martha Vaughan and Gustavo Pacheco-Rodriguez (National Heart, Lung, and Blood Institute [NHLBI], National Institutes of Health [NIH], Bethesda, MD, USA) for critical review of the manuscript and helpful discussions. This work was supported by the Intramural Research Program, NHLBI, NIH.

References
1. Ryu JH, Moss J, Beck GJ, Lee JC, Brown KK, Chapman JT, Finlay GA, Olson EJ, Ruoss SJ, Maurer JR, Raffin TA, Peavy HH, McCarthy K, Taveira-Dasilva A, McCormack FX, Avila NA, Decastro RM, Jacobs SS, Stylianou M, Fanburg BL: The NHLBI Lymphangioleiomyomatosis Registry: characteristics of 230 patients at enrollment. Am J Respir Crit Care Med 2006, 173:105-11.
2. Osborne JP, Fryer A, Webb D: Epidemiology of tuberous sclerosis. Ann NY Acad Sci 1991, 615:125-7.
3. Urban TJ, Lazor R, Lacroque J, Murriss M, Labrune S, Valeyre D, Cordier JF: Pulmonary lymphangioleiomyomatosis: a study of 69 patients. Medicine 1999, 78:321-37.
4. Smolarek TA, Wessner LL, McCormack FX, Mylet JC, Menon AG, Henske EP: Evidence that lymphangiomyomatosis is caused by TSC2 mutations: chromosome 16p13 loss of heterozygosity in angiomylipomas and lymph nodes from women with lymphangiomyomatosis. Am J Hum Genet 1998, 62:810-5.
5. Carsillo T, Astrinidis A, Henske EP: Mutations in the tuberous sclerosis complex gene TSC2 are a cause of sporadic pulmonary lymphangioleiomyomatosis. Proc Natl Acad Sci U S A 2000, 97:6085-90.
6. Brunelli A, Catalini G, Fanchini A: Pregnancy exacerabting unsuspected mediastinal lymphangioleiomyomatosis and chylothorax. Int J Gynaecol Obstet 1996, 52:289-90.
7. Yano S: Exacerbation of pulmonary lymphangioleiomyomatosis by exogenous oestrogen used for infertility treatment. Thorax 2002, 57:1085-6.
8. Taveira-DaSilva AM, Stylianou MP, Hedin CJ, Hathaway O, Moss J: Decline in lung function in patients with lymphangioleiomyomatosis treated with or without progesterone. Chest 2004, 126:1867-74.
9. Harari S, Cassandro R, Chiodini J, Taveira-DaSilva AM, Moss J: Effect of a gonadotrophin-releasing hormone analogue on lung function in lymphangioleiomyomatosis. Chest 2007, 133:448-54.
10. Goncharova EA, Krymskaya VP: Pulmonary lymphangioleiomyomatosis (LAM): progress and current challenges. J Cell Biochem 2008, 103:369-82.
11. Krymskaya VP, Goncharova EA: PI3K/mTORC1 activation in hamartoma syndromes: therapeutic prospects. Cell Cycle 2009, 8:403-13.
12. Lamb RF, Roy C, Diefenbach TJ, Vinters HV, Johnson MW, Jay DG, Hall A: The TSC1 tumour suppressor hamartin regulates cell adhesion through ERM proteins and the GTPase Rho. Nat Cell Biol 2002, 4:281-7.
13. Taveira-DaSilva AM, Steagall WK, Moss J: Lymphangioleiomyomatosis. Atlas of Genetics and Cytogenetics in Oncology and Haematology, October 2008. [http://atlasgeneticsoncology.org/Tumors/Lymphangioleiomyomatosis.html]
14. Polak P, Hall MN: mTOR and the control of whole body metabolism. Curr Opin Cell Biol 2009, 21:209-18.
15. Loewith R, Jacinto E, Wullschleger S, Lorberg A, Crespo JL, Bonenfant D, Opplyger W, Jenoe P, Hall MN: Two mTOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Mol Cell 2002, 10:437-48.
16. Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM: Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and
raptor-independent pathway that regulates the cytoskeleton. Curr Biol 2004, 14:1296-302.

F1000 Factor 6.6 Must Read
Evaluated by James Woodgett 04 Aug 2004, Thomas Neufeld 05 Aug 2004, Peter Taylor 20 Sep 2004

17. Huang J, Dibble CC, Matsuzaki M, Manning BD: The TSC1-TSC2 complex is required for proper activation of mTOR complex 2. Mol Cell Biol 2008, 28:1104-15.

F1000 Factor 3.0 Recommended
Evaluated by Robert Abraham 20 Jun 2008

18. Sarbassov DD, Guerin DA, Ali SM, Sabatini DM: Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 2005, 307:1098-101.

F1000 Factor 8.3 Exceptional
Evaluated by Norbert Perrimon 28 Feb 2005, Shiv Pillai 09 Mar 2005, Phillip Hawkins 16 Jun 2005, Sachdev Sidhu 05 Aug 2005

19. Roux PP, Baliff BA, Anjam R, Gygi SP, Blenis J: Tumor-promoting phorbol esters and activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6 kinase. Proc Natl Acad Sci U S A 2004, 101:13489-94.

20. Ma L, Chen Z, Erdjument-Bromage H, Tempst P, Pandolfo P: Phosphorylation and functional inactivation of TSC2 by Erk: implications for tuberous sclerosis and cancer pathogenesis. Cell 2005, 121:179-93.

F1000 Factor 3.2 Recommended
Evaluated by Michael Hall 16 May 2005, Angel Nebreda 23 May 2005

21. Inoke K, Li Y, Zhu T, Guan K-L: TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signaling. Nat Cell Biol 2002, 4:648-57.

22. Manning BD, Tee AR, Logson MN, Blenis J, Cantley LC: Identification of tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/Akt pathway. Mol Cell Biol 2002, 10:151-62.

23. Castro AF, Rebben JF, Clark GJ, Quilliam LA: Rheb binds tuberous sclerosis complex 2 (TSC2) and promotes S6 kinase activation in a rapamycin- and farnesylation-dependent manner. J Biol Chem 2003, 278:32493-6.

24. Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J: Tuberous sclerosis complex gene products, tuberin and hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. Curr Biol 2003, 13:1259-68.

25. Zhang Y, Gao X, Saucedo LJ, Ru B, Edgar BA, Pan D: Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. Nat Cell Biol 2003, 5:578-81.

F1000 Factor 6.0 Must Read
Evaluated by Thomas Neufeld 23 Jun 2003

26. Fingar DC, Salama S, Tsou C, Harlow E, Blenis J: Mammalian cell size is controlled by mTOR and its downstream targets S6K1 and 4EBP1/eIF4E. Genes Dev 2002, 16:1472-87.

F1000 Factor 4.8 Must Read
Evaluated by Ron Prywes 16 Jul 2002, Michael Hall 11 Sep 2002

27. Carriere A, Cargnello M, Julien L-A, Gao H, Bonnell E, Thibault P, Roux PP: Oncogenec MAPK signaling stimulates mTORC1 activity by promoting RSK-mediated raptor phosphorylation. Curr Biol 2008, 18:1269-77.

28. Haar EV, Lee S, Bandhakavi S, Griffin TJ, Kim D-H: Insulin signaling to mTOR mediated by the Akt/PKB substrate PRAS40. Nat Cell Biol 2007, 9:316-23.

29. Inoke K, Zhu T, Guan K-L: TSC2 mediates cellular energy response to control cell growth and survival. Cell 2003, 115:577-90.

F1000 Factor 4.8 Must Read
Evaluated by Iswar Harinaran 03 Dec 2003, Angus Nairn 09 Dec 2003

30. DeYoung MP, Horak P, Sofer A, Sgroi D, Ellisen LW: Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14-3-3 shuttling. Genes Dev 2008, 22:239-51.

F1000 Factor 3.2 Recommended
Evaluated by Robert Abraham 30 Jan 2008, Silvio Gutkind 12 Feb 2008

31. Sancak Y, Peterson TR, Shaul YD, Lindeque RS, Thoreen CC, Bar-Peled L, Sabatini DM: The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science 2008, 320:1496-501.

F1000 Factor 6.0 Must Read
Evaluated by John Kyriakis 19 Jun 2008

32. Brown EJ, Beal PA, Keitch CT, Chen J, Shin TB, Schreiber SL: Control of p70 s6 kinase by kinase activity of FRAP in vivo. Nature 1995, 377:441-6.

33. Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markard AH, Sabatini DM: Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell 2006, 22:159-68.

34. Zeng Z, Sarbassov dos D, Samueld JJ, Yee KW, Muenell MF, Ellen Jackson C, Gisles FJ, Sabatini DM, Andreiff M, Konopleva M: Rapamycin derivatives reduce mTORC2 signaling and inhibit Akt activation in AML. Blood 2007, 109:3509-12.

35. Foster DA: Phosphatidic acid signaling to mTOR: signals for the survival of human cancer cells. Biochim Biophys Acta 2009, 1791:495-55.

36. Toschi A, Lee E, Xu L, Garcia A, Gadir N, Foster DA: Regulation of mTORC1 and mTORC2 complex assembly by phosphatidic acid: competition with rapamycin. Mol Cell Biol 2009, 29:1411-20.

37. Kenerson H, Dunton TA, Yeung RS: Effects of rapamycin in the Eker rat model of tuberous sclerosis complex. Pediatr Res 2005, 57:67-75.

38. Bissler JJ, McCormack FX, Young LR, Elwing JM, Chuck G, Leonard JM, Schmitstorff VJ, Laor T, Brody AS, Bean J, Salisbury S, Franz DN: Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangioleiomyomatosis. N Engl J Med 2008, 358:140-51.

F1000 Factor 9.0 Exceptional
Evaluated by Joel Moss 14 Feb 2008

39. Davies DM, Johnson SR, Tattersfield AE, Kingswood JC, Cox JA, McCarty DL, Doyle T, Elmslie F, Sagar A, de Vries PJ, Sampson JR, Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, Lane H, Eker rat model of tuberous sclerosis complex. Pediatr Res 2005, 57:67-75.

40. Shah OJ, Wang Z, Hunter T: Inappropriate activation of the TSC/Rheb/mTOR/PI3K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies. Curr Biol 2004, 14:1650-6.

41. Harrington LS, Findlay GM, Gray A, Tolkacheva T, D. Wang Z, Hunter T, Hofmann F, Hicklin DJ, Ludwig DL, Baselga J, Rosen N: mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res 2006, 66:1501-8.

F1000 Factor 3.0 Recommended
Evaluated by Alex Toker 06 Feb 2006

42. Carracedo A, Ma L, Teruya-Feldstein J, Rojo F, Salmena L, Alimonti A, Ega A, Sasaki AT, Thomas G, Kozma SC, Papa A, Nardella C, Cantley LC, Baselga J, Pandolfo P: Inhibition of mTORC1 leads
to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. J Clin Invest 2008, 118: 3065-74.

44. Mi R, Ma J, Zhang D, Li L, Zhang H: Efficacy of combined inhibition of mTOR and ERK/MAPK pathways in treating a tuberous sclerosis complex cell model. J Genet Genomics 2009, 36:355-61.

45. Yu J, Astrandis A, Howard S, Henske EP: Estradiol and tamoxifen stimulate LAM-associated angiomylipoma cell growth and activate both genomic and nongenomic signaling pathways. Am J Physiol Lung Cell Mol Physiol 2004, 286:L94-70.

46. Yu JJ, Robb VA, Morrison TA, Ariazi EA, Karbowniczek M, Astrandis A, Wang C, Hernandez-Cuebas L, Seelhofer LP, Nicolas E, Hensley H, Jordan VC, Walker CL, Henske EP: Estron promotes the survival and pulmonary metastasis of tuberin-null cells. Proc Natl Acad Sci U S A 2009, 106:2635-40.

47. Glassberg MK, Elliot SJ, Fritz J, Catanzaro P, Potier M, Donahue R, Steetler-Stevenson W, Kari M: Activation of the estrogen receptor contributes to the progression of pulmonary lymphangiomyomatosis via matrix metalloproteinase-induced cell invasiveness. J Clin Endocrinol Metab 2008, 93:1625-33

48. Clements D, Asprey SL, McCulloch TA, Morris TA, Watson SA, Johnson SR: Analysis of the oestrogen response in an angiomyolipoma-derived xenograft model. Endocr Relat Cancer 2009, 16:59-72.

49. Matsui T, Takeda K, Yu Z-X, Travis WD, Moss J, Ferrans VJ: Role for activation of matrix metalloproteinases in the pathogenesis of pulmonary lymphangioleiomyomatosis. Arch Pathol Lab Med 2000, 124:367-72.

50. Krymskaya VP, Shipley JM: Lymphangiomyomatosis. A complex tale of serum response factor-mediated tissue inhibitor of metalloproteinase-3 regulation. Am J Respir Cell Mol Biol 2003, 28:546-50.

51. Gibelston-Beading S, Powers EA, Stamp-Cole M, Scott PS, Wallace TL, Copeland J, Petzold G, Mitchell M, Ledbetter S, Tominaga S, Fukuchi Y: Vascular endothelial growth factor-D is increased in serum of patients with lymphangioleiomyomatosi. Lymphat Res Biol 2006, 4:143-52.

52. Young LR, Inouye Y, McCormack FX: Diagnostic potential of serum VEGF-D for lymphangioleiomyomatosis. N Engl J Med 2008, 358:199-200.

53. Glassow CG, Avila NA, Lin JP, Stylianou MP, Moss J: Serum vascular endothelial growth factor-D levels in patients with lymphangioleiomyomatosis reflect lymphatic involvement. Chest 2009, 135:293-300.

54. Jaiswal VR, Baird J, Fleming J, Miller DS, Sharma S, Molberg K: Localization of the lymphangioleiomyomatosis mimicking malignancy. A case report and review of the literature. Arch Pathol Lab Med 2003, 127:879-82.

55. Su H-C, Wang J, Tsang Y-M, Lin M-C, Li Y-W: Lymphangiomyomatosis initially presenting with abdominal pain. A case report. J Clin Imag 2003, 27:166-70.

56. Wong YS, Yeung TK, Chu WC: Atypical presentation of lymphangioleiomyomatosis as acute abdomen: CT diagnosis. AJR Am J Roentgenol 2003, 181:284-5.

57. Ryu JH, Doerr CH, Fisher SD, Olson EJ, Sahn SA: Chylothorax in lymphangioleiomyomatosis. Chest 2003, 123:623-7.

58. Almossa KD, McCormack FX, Sahn SA: Pleural disease in lymphangioleiomyomatosis. Clin Chest Med 2006, 27: 355-68.

59. Kimura M, Morikawa T, Takeuchi K, Furuhi H, Fukumura R, Kakuta Y, Kamisumura S, Tashiro Y: Lymphangiomyomatosis with chylosic ascites therapy successfully by peritoneovenous shunting. Nihon Kyobu Shikkan Gakkai Zasshi 1996, 34:557-62.

60. Makino Y, Shimaniu Y, Fujuwara N, Morio Y, Sato K, Yoshimoto J, Gunji Y, Suzuki T, Sasaki S, Iwase A, Kawasaki S, Takahashi K, Seyama K: Peritoneovenous shunting for intractable chylosic ascites complicated with lymphangioleiomyomatosis. Intern Med 2008, 47:281-5.
78. Mikroulis D, Didilis V, Bitzikas G, Bougioukas G: Octreotide in the treatment of chylothorax. Chest 2002, 121:2079-80.

79. Makrilakis K, Pavlatos S, Giannikopoulos G, Toubanakis C, Katsilambros N: Successful octreotide treatment of chylothoracic effusion and lymphedema in the yellow nail syndrome. Ann Intern Med 2004, 141:246-7.

80. Bulbul A, Okan F, Nuhoglu A: Idiopathic congenital chylothorax presented with severe hydrops and treated with octreotide in term newborn. J Matern Fetal Neonatal Med 2009, 25:1-4.

81. Bissler JJ, Kingswood JC: Renal angiomyolipomata. Kidney Int 2004, 66:924-34.

82. Wong IY, Shortliffe LD: The management of renal angiomyolipomas in a patient with tuberous sclerosis. Nat Clin Pract Urol 2009, 6:168-72.

83. Sooriakumaran P, Gibbs P, Coughlin G, Attard V, Elmslie F, Kingswood C, Taylor J, Corbishley C, Patel U, Anderson C: Angiomyolipomata: challenges, solutions, and future prospects based on over 100 cases treated. BJU Int 2009, [Epub ahead of print].