Pulmonary Lymphangioleiomyomatosis

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Lymphangioleiomyomatosis cells were recently recognized as perivascular epithelioid cells, and LAM is categorized as one of the perivascular epithelioid cell tumor family, which includes angiomyolipomas, clear cell "sugar" tumors of the lung and extrapulmonary sites, clear cell myomelanocytic tumors of the fallopian ligament or ligamentum teres, and rare clear cell tumors of other anatomic sites. Proliferation and infiltration by LAM cells lead to the cystic destruction of the lung parenchyma; obstruction of airways, blood vessels, and lymphatics; and loss of pulmonary function. Lymphangioleiomyomatosis cells coexpress smooth muscle markers (such as smooth muscle actin and desmin) and melanocytic markers (such as HMB-45, Melan-A/MART-1, and microphthalmia transcription factor). Dyspnea on exertion and recurrent pneumothorax are the most common clinical features. Somatic or genetic mutations of tumor suppressor genes tuberous sclerosis complex (TSC) 1 or TSC2 are closely related to lymphangioleiomyomatosis. The TSC1/TSC2 protein-related signaling pathways are involved in the pathogenesis and may provide novel therapeutic targets for lymphangioleiomyomatosis and diseases associated with TSC1/TSC2 dysfunction.

(CLINICAL FEATURES AND RADIOLOGY)

Lymphangioleiomyomatosis occurs in 2 main forms: tuberous sclerosis complex (TSC)-associated LAM and sporadic LAM (S-LAM). Tuberous sclerosis complex, an autosomal dominant syndrome affecting 1 in 6000 newborns and approximately 1.5 million people worldwide, is characterized by hamartomatous involvement of the brain, kidney, skin, and eye and is also often associated with severe mental retardation, epilepsy, and autism. The prevalence of LAM among women with TSC is 26% to 39%, and TSC is present in 14.8% of patients with LAM. Most LAM cases are sporadic, and S-LAM represents about 85% of the patients in the National Heart, Lung, and Blood Institute LAM Registry and those registered with the LAM Foundation. Typically, LAM is a disease of women of childbearing age and worsens during pregnancy and following the administration of estrogen. The mean (SD) age at disease onset is 38.9 (0.73) years and at diagnosis, 41.0 (0.65) years. Rarely, LAM can be seen in men. The global prevalence of pulmonary LAM is unknown and is estimated at approximately 1 to 5 of 10 women. However, the true prevalence is likely greater because of misdiagnosis and subclinical disease.

Lymphangioleiomyomatosis is characterized clinically by dyspnea on exertion and recurrent pneumothorax. Less common clinical features include chest pain, cough, hemoptysis, chylothorax, and ascites. Chyloptysis, chyluria, chylous pericardial effusion, pneumoperitoneum, acute abdomen, and lymphedema have also been described. Symptoms have been reported to worsen during pregnancy. Renal angiomyolipomas are usually asymptomatic but may cause flank pain, hematuria, or a palpable mass. Spontaneous pneumothorax can be the presenting feature and is suggestive of LAM. For the 230 patients registered with National Heart, Lung, and Blood Institute LAM Registry, spontaneous pneumothorax was the sentinel event leading to the diagnosis of LAM in 35.8% of patients. Recently, Watz et al reported that the most frequently stated first clinical sign was spontaneous pneumothorax (37.5%). Physical examination findings may include crackles, wheezes, clubbing, pneumothorax, ascites, or signs of pleural effusion.

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myomatosis should be considered in women who present with dyspnea on exertion that is associated with pneumothorax, hemoptysis, or an abnormal finding on a chest radiograph.

The chest radiographic features of LAM have been well described. In the early stage of disease, chest radiographic findings may be normal. Subsequently, fine reticular or reticulonodular infiltrates develop. Pleural abnormalities, including pleural effusions and pneumothorax, are common findings. Unlike the chest radiograph, high-resolution computed tomography findings are almost always abnormal at the time of diagnosis, are a more sensitive indicator of early disease, and may show abnormalities in patients with normal findings on chest radiographs. The most common feature is the presence of numerous, thin-walled cysts. These cysts vary in size, from a few milliliters to several centimeters, and in number, from a few scattered cysts to a near-complete replacement of the lung parenchyma by cysts.

**PATHOLOGIC FEATURES**

**Macroscopic Features**

Gross pathologic examination of lungs with LAM reveals a cystic, honeycomb appearance. The cysts are usually evenly distributed throughout the lung and may contain air or fluid (serosanguinous or chylous). The lungs are enlarged as seen in severe emphysema. The cysts range from 0.5 to 2 cm and can be more than 10 cm.

**Microscopic Features**

In the early stage of the disease, the infiltrates by LAM cells may be overlooked, and the biopsy can be misinterpreted as showing either emphysema or healthy lung. The LAM cells are characteristically found in small clusters or nests at the edges of the cysts and along the alveolar walls, pulmonary blood vessels, lymphatics, and bronchioles (Figures 1 through 4). Mitotic figures are rarely seen. The infiltration of LAM cells in the walls of distal airways and vessels can lead to airway obstruction, air trapping, bullae formation, pneumothorax, hemoptysis, and foci of hemosiderosis. The intimate relationship of LAM cells to the lymphatic vessels is believed to be responsible for chylothorax and chylous ascites formation. Loss of alveoli is associated with cyst formation. The cyst walls contain LAM cells and are usually lined by alveolar and patches of bronchiolar epithelium (Figure 4). The proliferating LAM cells are morphologically heterogeneous and can be classified into 2 types: spindle-shaped cells and epithelioid cells. Usually, the spindle-shaped cells are centrally located, whereas the epithelioid cells exist in the peripheral regions of the LAM cell nodules (Figure 3). Juve et al proposed that the 2 subpopulations of LAM cells may represent sequential stages of differentiation downstream of a LAM stem cell. An alternate possibility is that the 2 cell types represent alternative phenotypes and that differentiation into one or the other phenotype is under the control of unknown stimuli.

**Immunohistochemistry**

Characteristically, proliferating LAM cells exhibit features of coexpressing contractile proteins (smooth muscle actin and desmin) and melanocytic markers (HMB-45, HMAS-1, Melan-A or MART-1, and microphthalmia transcription factor) (Figures 5 and 6). These features mean this lesion can be considered one of the perivascular epithelioid cell tumor family members. Interestingly, HMB-45 positivity is reversely related to cell proliferation. Spindle-shaped cells express less HMB-45 and more proliferating cell nuclear antigen. In contrast, epithelioid LAM cells exhibit more HMB-45 positivity with less proliferating cell nuclear antigen expression. Similar to proliferating cell nuclear antigen expression, the reaction to membrane type-1 matrix metalloproteinase (MMP) and MMP-activating enzymes are predominantly localized in spindle-shaped LAM cells. These data indicate that the spindle-shaped cells are in a more proliferative stage, and epithelioid cells are in a more mature state. Matsui et al demonstrated the presence of estrogen receptor (ER) and progesterone receptor (PR) in the epithelioid LAM cells from 5 of 10 patients (50%) who never received hormone treatment. However, no ER or PR positivity was observed in patients after receiving therapy with progesterone and tamoxifen. These data indicated that ER and PR are selectively expressed in epithelioid LAM cells and are down-regulated by hormone therapy. There is no clear evidence that patients with ER positivity are more likely to respond to hormonal manipulation. Recently, CD1a and cathepsin K were found to be positive in both spindle-shaped and epithelioid LAM cells, and they may provide useful new markers for the diagnosis of pulmonary LAM and renal angioleiomyoma.

**Ultrastructural Features**

Scanning electron microscopic analysis, in patients with LAM, reveals the dilation of the air spaces and the formation of cysts in the lungs, which are lined with type II pneumocytes. Transmission electron microscopic analysis shows actinlike filaments, peripherally arranged dense bodies, and premelanosome-like structures in the cytoplasm of LAM cells. The cells are surrounded by bundles of collagen.

**Cytology and Laboratory Findings**

Mitani et al recently evaluated LAM-associated chyloous effusions from patients with LAM, and demonstrated that the cytologic features of LAM cell cluster were a well-organized, globular cluster consisting of LAM cells enveloped by lymphatic endothelial cells. The LAM cells form a tightly cohesive core, have a moderate nuclear to cytoplasmic ratio, and are positive for muscular antigens, melanoma-related antigens, and PR staining. These data indicate that the cytologic and immunocytochemical examinations of chyloous effusions can be considered to have diagnostic significance for LAM. There is no specific laboratory abnormality associated with LAM. One study measured serum levels of vascular endothelial growth factor (VEGF)-A, VEGF-C, and VEGF-D in patients with LAM and found only VEGF-D was significantly increased. The elevated serum VEGF-D levels may be a valuable surrogate marker for evaluating the disease severity in LAM. Immunohistochemical studies have suggested that excess MMPs, synthesized by LAM cells, function in the proteolytic mechanisms of this disease. Odajima et al collected serum samples from patients with LAM and found serum levels of MMP-9,
Figure 1. Histologic section showing cystic spaces lined by thickened interstitium (hematoxylin-eosin, original magnification ×40).

Figure 2. Histologic section showing nodules of lymphangioleiomyomatosis cells (hematoxylin-eosin, original magnification ×100).

Figure 3. Histologic section showing nodules composed of central, spindle-shaped, and peripheral epithelioid lymphangioleiomyomatosis cells (hematoxylin-eosin, original magnification ×200).

Figure 4. Histologic section showing a cystic wall lined by lymphangioleiomyomatosis cells and adjacent alveolar, hemosiderin-laden macrophages (hematoxylin-eosin, original magnification ×100).

Figure 5. Immunohistochemical staining showing lymphangioleiomyomatosis cell immunoreactivity for smooth muscle actin (original magnification ×100).

Figure 6. Immunohistochemical staining showing lymphangioleiomyomatosis cell immunoreactivity for HMB-45 (original magnification ×200).
but not MMP-2, were significantly elevated in the patients with LAM compared with controls.

DIFFERENTIAL DIAGNOSIS

Lymphangioleiomyomatosis can mimic several lesions clinically, radiographically, and pathologically. Emphysema presents with enlarged lungs and cystic airspaces, but it lacks LAM cells, and LAM tends to have better-defined and more-uniform cystic air spaces. Langerhans cell histiocytosis can present with cyst formation radiographically and with spontaneous pneumothorax clinically. However, the predilection of the small nodules or nodules with cystic changes in the mid- and upper-lung zones and the infiltrating Langerhans cell (positive for CD1a, S100, and langerin) and eosinophils will differentiate it from LAM. Benign metastasizing leiomyoma is not usually associated with cysts, and the nodules of smooth muscle are generally larger than those seen in LAM. Patients always have a history of uterine leiomyoma, and the smooth muscle cells are negative for melanocytic markers, such as HMB-45 and Melan-A. Diffuse pulmonary lymphangiomatosis shows diffuse proliferation of lymphatic vascular spaces and smooth muscles, mimicking LAM. The disease usually affects children of either sex rather than women of childbearing age. Patients with diffuse pulmonary lymphangiomatosis can develop interstitial infiltrates and pleural and pericardial effusions, and histologically, anastomosing endothelial-lined spaces are seen along the pulmonary lymphatic routes, pleura, and mediastinum. Compared with LAM, the smooth muscle proliferation is less marked, without extension into the alveoli or cyst formation, and is negative for HMB-45 staining.10 Minute pulmonary meningoential nodules are lesions histologically composed of small nests of epithelioid cells located within the interstitium of the lung. These nodules are generally asymptomatic and are usually found incidentally in resected lungs with malignant pulmonary tumors. However, minute meningoential nodules can be disseminated with bilateral pulmonary involvement (diffuse pulmonary meningoletalomatosis) and is associated with clinical symptoms of restrictive pulmonary disease and radiologic evidence of diffuse reticulonodular pulmonary infiltrates, which may mimic LAM. Histologically, the lesions of minute pulmonary meningoletal are composed of small clusters of epithelioid cells, with round to oval nuclei devoid of atypia and surrounded by abundant eosinophilic cytoplasm. Immunohistochemical studies show positive staining for epithelial membrane antigen, vimentin, CD56, and PR, but negative staining for cytokeratin, smooth muscle actin, S100, CD34, chromogranin, synaptophysin, and HMB-45.20,21 The absence of cyst formation, HMB-45 positivity, and the presence of chronic lung diseases or a malignant pulmonary tumor in the background help distinguish this entity from LAM.

PATHOGENESIS

Origin of LAM cells

Because of the coexpression of contractile proteins (smooth muscle actin and desmin) and melanocytic markers (HMB-45, and Melan-A or MART-1), LAM cells are suggested to be of perivascular epithelioid cell origin. However, the origin of LAM cells is still unclear. Initially, LAM cells were believed to be derived from either airway or vascular smooth muscle cells, but this hypothesis is not supported by the diffuse existence of LAM cells throughout the lungs and the irregular distribution within the nodules, without the formation of organized layers. Another hypothesis, based on some clinical, genetic, and cell culture studies, is that LAM cells can originate from angiomyolipoma and be brought into the lungs. Lymphangioleiomyomatosis cells have been found in the blood, urine, and chylous fluids of patients with LAM,18 indicating that LAM cells can leave the primary lesions, disseminate through blood or lymph vessels, and implant into secondary sites.2,24 Indeed, recurrence of LAM cells after lung transplantation has occurred in patients with LAM.22,26 and the LAM cells in the allograft are derived from the patient’s original LAM cells.25 Identical TSC2 gene mutations were also detected in pulmonary LAM cells and in angiomyolipoma cells from renal tumors of patients with TSC and LAM.27 Pulmonary LAM cells may represent metastasized secondary tumors. However, about one-third of S-LAM cases are without angiomyolipoma, and in such cases, LAM cell origin cannot be explained by metastatic or neoplastic cell dissemination. Another hypothesis about the origin of LAM cells was that they began as donor cells from a nonhost source. The source of nonhost cells could be an organ donor, a blood transfusion, or fetal cells persisting in maternal circulation.15 Evidence for the nonhost cell theory still remains elusive.

LAM and TSC

The recognition of the similarity between the pulmonary lesions seen in otherwise healthy women with LAM, and those seen in patients with TSC and lung involvement has led to the hypothesis that TSC-associated LAM and S-LAM might share common pathogenetic mechanisms.1 The tumor suppressor genes TSCI and TSC2 are closely related to the pathogenesis of LAM and the proliferation of LAM cells. Mutations in TSC2 gene arise more frequently (in most LAM cases and in about 60% of TSC cases) than TSCI mutations.16,24 The prevailing model for LAM is that the disease develops through a 2-hit mechanism: a mutation in either the TSCI or TSC2 gene, followed by a second hit referred to as a loss of heterozygosity, leading to a loss of function of either the TSCI or TSC2 proteins. Thus, S-LAM develops from 2 acquired mutations (predominantly in TSC2), and patients with TSC-associated LAM have one germline mutation and one acquired mutation (again, predominantly in TSC2). These findings explain why LAM occurs frequently in patients with TSC, whereas S-LAM is uncommon.19 Mutational analysis of familial and sporadic cases of TSC revealed that a TSC2 mutation is associated with disease severity compared with TSCI mutations.23,24 Most recently, Muzykewicz et al25 conducted a retrospective review and found the predominant size of cysts did not differ between patients with either the TSCI or the TSC2 mutation, but women with a TSC2 mutation and LAM had more cysts than did patients with a TSCI mutation.

TSCI gene is located on the long arm of chromosome 9 (9q34) and encodes the protein hamartin (130 kDa), whereas TSC2 is located on the short arm of chromosome 16 (16p13.3) and encodes the protein tuberin (198 kDa).
These proteins are conserved and ubiquitously expressed. Hamartin and tuberin form a physical and functional heterodimer complex, in which hamartin functions as the regulatory component, stabilizing tuberin and facilitating the tuberin catalytic function as a GTPase-activating protein. Tuberin, which is assumed to be the functional component of this protein complex, is multifunctional, and involved in the regulation of cell size, cell cycles, translation, transcription, apoptosis, and cell differentiation. A major function of the TSC1 (hamartin)–TSC2 (tuberin) complex is its role as a GTPase-activating protein against Rheb (Ras homolog enriched in brain), which in turn regulates mTOR (mammalian target of rapamycin) signaling. The major function of mTOR is to phosphorylate and activate downstream targets p70S6K (p70 ribosomal protein S6 kinase) and 4E-BP1. Deficiency or dysfunction of the encoded proteins, hamartin or tuberin, respectively, result in constitutive activation of mTOR and downstream S6K and 4E-BP1, leading to increased protein translation and, ultimately, to inappropriate cellular proliferation, migration, and invasion. Tuberin is phosphorylated by several factors and signaling pathways, such as growth factors, cellular energy and nutrient levels, the PI3K-Akt pathway, the ERK 1/2-RSK pathway, the Jak-STAT3 pathway, the oxygen-sensing (hypoxia) pathway, and the Wnt pathway. The TSC1/TSC2-related signaling pathways have also been found to be involved in the pathogenesis of LAM and may provide novel therapeutic targets for LAM and for diseases associated with TSC1/TSC2 dysfunction.24,29

TREATMENT

Lymphangioleiomyomatosis is predominantly a disease of premenopausal women and can worsen during pregnancy and following the administration of estrogens. These observations and the finding that LAM tissues express ER and PR have led to several hormone-based treatments, such as bilateral oophorectomy, progesterone.31 Schiavina et al treated 36 patients with LAM with hormonal therapy for 20 years and found hormonal therapy had the capability of reducing mortality and improving the quality of life. The survival rate of patients in their study since clinical onset was 97% at 5 years, 90% at 10 years, and 71% at 25 years. However, the efficacy of hormone therapy is still controversial. In patients with airflow obstruction, inhaled bronchodilator therapy may provide symptomatic relief. Lung transplantation has been accepted as a therapy for end-stage pulmonary LAM, and the 1, 2, 5, and 10 year survival rates after transplant are 79.6%, 74.4%, 64.7%, and 52.4%, respectively.26 Remarkable advances in the genetics of TSC and knowledge of the cellular signaling pathways modulated by hamartin and tuberin have enhanced the understanding of the biology of LAM and have provided several potential therapeutic targets currently being studied. Novel macrolide agent sirolimus (rapamycin), acting as an mTOR inhibitor, is used as an immunosuppressive agent in solid organ transplantation and as an antiproliferative agent for the treatment of various types of cancer; it has shown promising results in the treatment of LAM.20 Other therapeutic targets, such as MMP inhibition by MMP inhibitor (doxycycline), RHd GTPase inhibition by 3-hydroxy-3-methylglutaryl-coenzyme A inhibitors (statins), or JAK-STAT3 pathway inhibition by interferon-γ, may also have potential for the treatment of LAM.4

PROGNOSIS

The natural history of LAM is progressive airflow restriction leading to respiratory failure and cor pulmonale. However, the rate of progression varies among patients, and there are no widely accepted clinical and pathologic predictors of disease rapid progression. The reported 5-year survival ranges from 50% to 97%.2,6 The large range and disparity in survival data may reflect the small numbers of patients with LAM included in the reports, the variability of clinical characteristics, and the different treatments.

References


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