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Lymphangioleiomyomatosis (LAM): Molecular insights lead to targeted therapies

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Summary
LAM is a rare lung disease, found primarily in women of childbearing age, characterized by cystic lung destruction and abdominal tumors (e.g., renal angiomyolipoma, lymphangioleiomyoma). The disease results from proliferation of a neoplastic cell, termed the LAM cell, which has mutations in either of the *tuberous sclerosis complex (TSC) 1* or *TSC2* genes. Molecular phenotyping of LAM patients resulted in the identification of therapeutic targets for drug trials. Loss of *TSC* gene function leads to activation of mammalian target of rapamycin (mTOR), and thereby, effects on cell size and number. The involvement of mTOR in LAM pathogenesis is the basis for initiation of therapeutic trials of mTOR inhibitors (e.g., sirolimus). Occurrence of LAM essentially entirely in women is consistent with the hypothesis that anti-estrogen agents might prevent disease progression (e.g., gonadotropin-releasing hormone analogues). Levels of urinary matrix metalloproteinases (MMPs) were elevated in LAM patients, and MMPs were found in LAM lung nodules. In part because of these observations, effects of doxycycline, an anti-MMP, and anti-angiogenic agent, are under investigation. The metastatic properties of LAM

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cells offer additional potential for targets. Thus, insights into the molecular and biological properties of LAM cells and molecular phenotyping of patients with LAM have led to clinical trials of targeted therapies. Funded by the Intramural Research Program, NIH/NHLBI

Lymphangioleiomyomatosis (LAM) is a rare disease that affects primarily women of childbearing age[1–5] with an incidence of approximately 2.6 per 1 million women.[3] Organs involved in LAM include the lung, kidney (e.g., angiomyolipomas (AMLs)), and axial lymphatics (e.g., lymphangioleiomyomas, adenopathy).[1–5] Patients with LAM present most frequently with dyspnea and pneumothorax[1–5]; other symptoms evident at presentation or during the course of the disease include chylothorax, hemoptysis, and ascites.[1–3,5] Patients usually first notice symptoms in their mid to late 30s,[2–5] although diagnosis may be delayed for 5—6 years, in part due to similarities of the symptoms to those of more common lung diseases. Survival rate 10 years after diagnosis was approximately 85%[6] or 90%.[7] The frequency of LAM is elevated among patients with tuberous sclerosis complex (TSC), an inherited disorder resulting from mutations in either the *TSC1* or *TSC2* genes. The clinical phenotype results from proliferation of the neoplastic LAM cell, which contains the TSC mutation.

Characteristics of LAM

Pulmonary dysfunction

The characteristic pulmonary radiologic (computed tomography (CT)) finding in patients with LAM is thin-walled cysts spread diffusely throughout the lung parenchyma, with no apical or basilar dominance.[1,5,6,8] Cystic changes, or the proliferation of LAM cells, are responsible for airflow obstruction and decreased lung diffusion capacity. FEV_1 (forced expiratory volume in one second) and DLco (diffusion capacity of the lungs for carbon monoxide) are reduced in approximately 60% of patients.[8] Evidence of air-trapping was observed on ventilation-perfusion scintigrams.[2,9]

Pathology

LAM results from the proliferation of abnormal smooth muscle-like cells (LAM cells), neoplastic cells that contain smooth muscle (α-smooth muscle actin (α-SMA)) and melanoma cell (gp100) antigens[10] as well as tuberous sclerosis complex (*TSC)* gene mutations.[11–13] Immunohistochemistry revealed two morphological types of LAM cells: epithelioid cells that react with HMB-45 (a monoclonal antibody that recognizes a splice variant of pmel-17 (gp100)) and spindle-shaped cells reactive with antibodies against proliferating cell nuclear antigen.[14] LAM cells proliferate in the vicinity of blood and lymphatic vessels, near bronchioles, and in walls of the cystic lesions.[10] In the lung, LAM cell nodules in the lung are traversed by slit-like lymphatic channels, whereas in extrapulmonary lesions, LAM cells are seen in fascicles, which form plump rod-shaped bundles separated by lymphatic channels.[15] Lung and lymphatic lesions and AMLs contain smooth muscle-like LAM cells. AMLs differ, however, from other LAM lesions in that they contain also underdeveloped vasculature and adipose tissue.[10,15–17] Hyperplastic type II pneumocytes are seen in LAM lung nodules.[18]

Pathogenesis

TSC is an autosomal dominant disorder, characterized by hamartomatous lesions, seizures, and mental retardation,[19] resulting from mutations in the *TSC1* or *TSC2* genes, which encode hamartin or tuberin, respectively.[20] Approximately one-third of women with TSC will present with pulmonary cystic lesions radiographically and histologically identical to those in LAM.[21–23] *TSC2* mutations are much more frequent than those of *TSC1* in sporadic LAM patients.[11–13] In patients with LAM, who have received a lung transplant, *TSC* mutations identical to those present in the explanted lung were observed in the donor lung, consistent with metastatic properties of LAM cells.[24,25] Similarly consistent with a metastatic model of disease progression, LAM cells were also isolated from blood, urine, and chyle of LAM patients.[26]

LAM natural history study

More than 500 patients with LAM, primarily from the United States and Canada, but also from Europe and Southeast Asia, were enrolled in the LAM natural history protocol (NHLBI protocol 95-H-0186). In this longitudinal study, over 250 patients returned for five or more visits. Data on survival and disease progression from X-ray, biopsy, and/or physiological (e.g., pulmonary function tests) procedures were generated and collated.[5]

Predictors of time to death or transplantation

LAM histology scores

Severity of lung involvement in LAM was assessed in patients' lung biopsies using the LAM Histology Score (LHS). LHS is based on the extent of replacement of normal lung tissue by cystic lesions and LAM cell infiltrates.[27] The total percentage of tissue involvement by these two histologic patterns is graded as follows: LHS-1, $<$25%; LHS-2, 25% to 50%; and LHS-3, $>$50% of lung tissue involved. Using this grading method, significant differences in survival and time to transplantation for patients with LHS-1, -2, and -3 scores were observed (Fig. 1). The ten-year survival was found to be near 100% for LHS 1, 74.4% for LHS 2, and 52.3% for LHS 3.[27] These data confirmed prior observations showing that patients with more cystic disease have worse prognosis, and are more likely to have lower DLco and more exercise-induced hypoxemia than those with more muscular, solid lesions.[1] There was also a good correlation between DLco and FEV_1 and LHS[8] (Fig. 2).