LYMPHANGIOLEIOMYOMATOSIS (LAM) is a rare disease that affects females of reproductive age (38). Pathologically, it is characterized by the abnormal proliferation of immature smooth muscle-like cells that grow aberrantly in the lung. The relentless growth of LAM cells in the pulmonary airway, parenchyma, lymphatics, and blood vessels leads to respiratory failure and death. Recent progress has been made in understanding more about the pathogenesis of LAM. Studies such as those published by Yu et al., one of the current articles in focus (Ref. 42, see page L694 in this issue), deepen our understanding of the disease and facilitate the identification of potential therapeutic targets aimed at abrogating the aberrant cellular proliferation characteristic of LAM.

The LAM Cell: What Is It?

To understand the pathogenesis of LAM, one needs to have a keen understanding of the nature of the atypical cell so characteristic of LAM. In 1966, Cornog and Enterline (8) described the first significant collection of patients with LAM. These insightful observers postulated that the cell present in LAM lung was smooth muscle like, lacked the features of a malignancy, and perhaps shared a common genetic origin. Indeed, the cells found in LAM are not malignant and stain positive for smooth muscle markers, α-smooth muscle actin, desmin, and vimentin (9). What is unusual about LAM is that it is not due to the proliferation of one monoclonal cell type only but instead due to the infiltration and proliferation of a phenotypically heterogeneous group of smooth muscle cells, many of which have myofibroblast- and epithelioid-like features (4). Interestingly, LAM cells are unusually reactive to mouse monoclonal antibody HMB45 (20a), which reacts with a glycoprotein (gp100) found in immature melanocytes. However, considerable variability of HMB45 positivity is frequently observed and is not absolutely necessary for the diagnosis of LAM (20a, 30). Although the relevance of HMB45 positivity and its antigen gp100 is as yet unstudied, an inverse relationship has been described in LAM smooth muscle cells between immunostaining for HMB45 and proliferating cell nuclear antigen (PCNA), which is a marker of cell proliferation (30). Thin, spindle-shaped smooth muscle cells are less likely to be immunoreactive for HMB45 and more likely to be PCNA positive. In contrast, larger epithelioid-like smooth muscle cells are more likely to exhibit positive immunostaining for HMB45 with poor or no reactivity for PCNA. These observations have led to the hypothesis that LAM cells are a population of smooth muscle cells that at any one point in time may be in a state of variable differentiation (22). Furthermore, it is reasonable to postulate that each phenotype is associated with a cell-specific function, at least in terms of its proliferative capacity, which may help to explain why many patients with LAM have variable responses to therapy and outcomes.

Cornog and Enterline (8) also postulated in 1966 that the smooth muscle cells may share a common genetic origin. They noted that pulmonary LAM is histologically indistinct from the pulmonary lesions observed in tuberous sclerosis complex (TSC), and recent reports indicate that LAM has a prevalence as high as 39% in TSC (10, 31, 32). TSC, which is characterized by hamartomatous proliferations in the brain, kidney, skin, heart, and lung, is caused by germline mutations of either the TSC1 or TSC2 gene located on chromosomes 9q34 and 16p13, respectively (18). It is now clear that both of these genes are tumor suppressor genes encoding hamartin (TSC1) and tuberin (TSC2) and that the tumors associated with TSC have undergone loss of heterozygosity (LOH), indicating that tumor development in TSC is associated with the “two-hit” hypothesis of Knudson (5). In 1998, the discovery of LOH of the TSC2 gene in the angiomylolipomas (AMLs) and lymph nodes of women who suffer from sporadic LAM heralded a new wave of investigation of the role of the TSC genes and, in particular, the TSC2 gene in sporadic LAM (36). Subsequently, mutational analysis of the 41 coding exons of the TSC2 gene from LAM patients revealed that germline mutations of the TSC2 or TSC1 genes are extremely uncommon (1). Additional analysis of microdissected smooth muscle cells from renal AMLs and pulmonary LAM lesions revealed that mutations of the TSC2 gene in renal and pulmonary smooth muscle cells are not found in surrounding normal kidney or lung (6). Subsequently, single-strand conformation polymorphism (SSCP) analysis revealed that a spectrum of TSC2 gene mutations exists in microdissected smooth muscle cells from sporadic pulmonary LAM (37). These studies support the hypothesis that LAM is caused by a somatic mutation in the TSC2 gene. However, mutations of the TSC2 gene were not detected in every patient. This may be due to limitations of SSCP in the detection of important mutations, contamination of the microdissected specimen with other pulmonary cells that may not express mutations of TSC2, or perhaps inconsistencies of mutations of the TSC2 gene in LAM cells and LAM patients. Nonetheless, despite these limitations, the available evidence is convincing for a strong association between somatic TSC2 gene mutations and sporadic LAM.

The LAM Cell: Where Does It Come From?

Studies supporting a causative role for somatic mutations of the TSC2 gene in sporadic LAM (6, 37) suggest that a normal resident pulmonary cell undergoes a spontaneous mutation resulting in aberrant proliferation locally. The question is which smooth muscle cell underwent such a mutation? In the lung, smooth muscle is predominantly found in the airway and vasculature with a small amount in lung interstitium. Based on the frequent observation of microscopic hemorrhage in LAM lesions, and the frequent involvement of blood vessels, it has been hypothesized that LAM cells may be derived from vas-
cular smooth muscle. It has been shown that four distinct phenotypes of smooth muscle exist in the media of bovine aorta and that each phenotype is associated with a specific gene expression profile (40). Interestingly, in culture, each smooth muscle cell phenotype has different proliferative capacities (15). Similarly, in LAM, distinct smooth muscle cell phenotypes also occur that express markers indicating phenotype differences in growth potential. These parallel observations between vascular and LAM smooth muscle cell biology have led to the hypothesis that the LAM smooth muscle cell may be derived from vascular smooth muscle that differentiates into a unique migratory or proliferative phenotype by undergoing a spontaneous mutation within the vessel wall. However, no direct evidence supports this theory, leaving it yet to be proven.

LAM is a multisystem disease that is not necessarily limited to lung involvement but can also involve lymph nodes, kidney, and colon. Recently, the observation that identical mutations are shared between the renal and pulmonary lesions in LAM have led some members of the LAM scientific community to hypothesize that the pulmonary LAM cell may have originally metastasized from a distant site (41). AMLs are highly vascular tumors that occur frequently in LAM (3, 24). They contain strong vascular and smooth muscle elements with histological appearances similar to pulmonary LAM and are also immunoreactive to HMB45 (7). AMLs that occur in sporadic pulmonary LAM share identical mutations of the TSC2 gene, indicating that they are genetically related. Karbowniczek et al. (23) have recently demonstrated five morphologically distinct vessel phenotypes in renal AMLs from patients with LAM, four of which were neoplastic in character, indicating a higher propensity to metastasize. In addition, smooth muscle cells are aberrantly found in the lung at autopsy in trauma patients (personal communication from L. S. Schuger), and on occasion, benign uterine leiomyomata can metastasize to the lung, resulting in a clinical syndrome similar to LAM (12). Thus it could be postulated that pulmonary LAM is a rare benign metastasizing disease.

An alternative, but closely allied, explanation for the aberrant proliferation of immature smooth muscle cells in the lung is that LAM cells are donor cells from a nonhost source. Recent studies indicate that cell traffic occurs between the fetus and mother during pregnancy and that low numbers of cells or DNA persist in the maternal circulation for years thereafter (27). This process, known as microchimerism, is more common in diseases that have a female prevalence, such as autoimmune diseases, but can also be found in other conditions, such as in transplant or transfusion patients, where the source of nonhost cells is derived from the organ donor or blood (11). LAM has a predilection to the female sex, but the role of microchimerism is as yet unstudied in LAM, and the true source of the LAM cell remains elusive.

The LAM Cell: How Does It Grow?

Investigating LAM at a cellular level has been hampered by the lack of agreement on what exactly constitutes a LAM cell and the absence of an animal model of the disease. As previously discussed, the LAM cell is not a single phenotype, but many phenotypes, with cell-specific protein expression profiles and differential proliferative capacities. Thus applying the technique of microdissection to LAM tissue allows the limited evaluation of one cell type only. One group of microdissected cells may behave very differently from 1) microdissected cells from another LAM lesion within the same patient or 2) microdissected cells from another patient. Furthermore, contamination from nearby cells, influence of stage of disease, and hormonal therapy may also interfere with the genetic makeup and phenotype of microdissected pulmonary cells from the lung. This leads to a lack of standardization in the scientific approach to the cellular investigation of LAM.

Nonetheless, one recent study clearly demonstrated activation of S6, a ribosomal protein intricately involved in regulating the cell cycle, in smooth muscle cells that were microdissected from LAM lung (19). These cells expressed an aberrant form of TSC2. Furthermore, reexpression of tuberin, the protein product of TSC2, in LAM-derived smooth muscle cells, led to reduced proliferation of LAM cells and reduced phosphorylation of S6. This is the first study to suggest that expression of mutant TSC2 in LAM smooth muscle cells leads to the aberrant cellular proliferation observed in LAM lung. Furthermore, the findings in this study parallel the observations of others investigating TSC2 biology who have noted that tuberin antagonizes the downstream pathways activated by the phosphorylation of tyrosine kinase receptors (16, 17, 33, 34). An explosion of research in the last five years has demonstrated that tuberin interacts with and binds to TOR (target of rapamycin), a cellular protein that is frequently activated in response to growth factor stimulation. Activation of mammalian TOR subsequently results in the downstream activation and phosphorylation of S6 (25). The study by Goncharova et al. (19) indicates that similar pathways are overactivated in LAM and suggests that studying the regulation of signaling pathways by tuberin provides useful insights into how LAM cells grow.

Because of the paucity of LAM tissue and the absence of an ideal cell line in which to study signaling pathways in LAM, many investigators have relied on alternative cellular models. Such models include that described by Yu et al. (42). Renal AML smooth muscle cells bear remarkable morphological similarity to pulmonary LAM smooth muscle cells. Yu et al. describe the development of a cell line from an AML from a LAM patient that expressed inactivating mutations in both alleles of the TSC2 gene. Importantly, they demonstrate that similar to pulmonary LAM cells, the estrogen receptors (ER) α and ERβ are aberrantly expressed in LAM-derived renal AML cells. Furthermore, these cells grow in response to hormonal stimulation, and hormone-mediated growth of LAM-associated renal AML cell activates potent mediators of cell growth, i.e., ERK and c-myc. This study closely parallels findings by other groups that demonstrated a positive growth response to estrogen in smooth muscle cells that are null for TSC2 and TSC1 genes (14, 26). Yu et al. (42) add to our understanding of the proliferative effect of estrogen in cells that express dysfunctional TSC proteins that contrasts the inhibitory growth effects of estrogen frequently observed in vascular smooth cells that constitutively express tuberin (13, 14). Of even greater significance, Yu et al. also demonstrate that tamoxifen is equal to estrogen in its potency as an activator of growth of LAM-derived AML cells. This observation supports the known fact that tamoxifen and, in some cases, progesterone, act as both estrogen agonists and antagonists and raises the question of whether anti-estrogen therapy is appropriate in LAM. Although estrogen has been implicated in the growth of LAM.
cells in vivo, it has not yet been demonstrated that pulmonary LAM-derived smooth muscle cells grow in culture in response to estrogen. However, if one extrapolates from the study of Yu et al. to the LAM patient, hormone-manipulating agents are potentially harmful and perhaps caution should be exercised when prescribing “anti-estrogen” therapy to patients who suffer from LAM.

The aberrant expression of the ER and growth factors and their receptors in the lungs of LAM patients has received attention by researchers. Interestingly, immunohistochemical studies show that IGF binding proteins-2, -4, and -6 are associated with spindle-shaped LAM cells, whereas type 5 IGF binding protein is associated with epithelioid LAM cells (39). Similarly, FGF receptor-2 (FGFR-2; bek) is primarily expressed in LAM smooth muscle cells compared with FGFR-1 (Flg), which is expressed in epithelial cells adjacent to LAM smooth muscle cells (21). Matsui et al. (29) demonstrated that ER, localized in the nuclei of epithelioid (nonproliferating) LAM cells, is downregulated by hormonal therapy. In contrast, spindle-shaped (proliferating) LAM cells express little ER but highly express membrane type 1 matrix metalloproteinase (MT-1 MMP) (29). MT-1 MMP is a membrane-associated protein that activates MMP-2. MMP-2 is a potent matrix-degrading enzyme that is upregulated in LAM lung (20, 28). MMPs regulate matrix turnover, but it has also been shown that MMPs can induce cell growth by the activation of IGF binding proteins (35). These studies suggest that differential expression of growth factors and their receptors in LAM may result in cell-specific functions to exert fibrogenic, proliferative, and matrix-regulating effects in LAM. The immunohistochemical analysis of LAM tissue sheds new insights into the potential roles of ER, growth factors, and MMPs in LAM. However, it is limiting in terms of translating the role of such proteins into cellular function. The roles of ER, growth factors, their receptors, and MMPs in LAM-derived cells in culture has not been studied and would be extremely useful in clarifying the complex role of each individual growth factor in the pathogenesis of LAM.

In summary, considerable progress has been made in our understanding of the pathogenesis of LAM. Although the jury is out on the true source of LAM cells, it is clear that they are a heterogeneous population of smooth muscle-like cells that variably exhibit immunoreactivity to HMB45. Evidence suggests that they possess specific functions regarding cell growth and matrix-degrading capacity and that the growth of at least a subpopulation of LAM cells is associated with the mutant expression of the TSC2 gene. Until a deeper understanding of the exact nature of LAM cells is achieved or until an animal model becomes available, scientists studying LAM will need to rely on immunohistochemical analysis of LAM tissue, the study of LAM-derived smooth muscle cells, renal AMLs, or other cells expressing aberrant forms of TSC2. However, it should be realized that limiting studies in LAM to specific genotypes may ultimately hamper the investigation of the pathogenesis of this disease. Studies that focus on TSC2 and TSC1 biology, other potential genetic mutations in LAM, the function of individual subpopulations of LAM cells, and factors that govern cell growth and matrix degradation all bear relevance to the pathogenesis of LAM.

REFERENCES


