Needle in the Haystack: Finding the Elusive Lymphangioleiomyomatosis Cell

In this issue of the *Journal*, Guo and colleagues (pp. 1373–1387) (1) use state-of-the-art single-cell techniques to provide insights into lymphangioleiomyomatosis (LAM), a rare multisystem disease primarily affecting women, which is characterized by cystic destruction of the lungs, kidney angiomyolipomas, and/or lymphatic involvement (2). LAM can occur sporadically or in conjunction with tuberous sclerosis complex (TSC), an autosomal-dominant disorder characterized by hamartomatous growths in the central nervous system, skin, heart, liver, and eyes. Cystic destruction of the lung is caused by the neoplastic LAM cell, an abnormal smooth muscle–like cell expressing melanocyte proteins and having inactivating mutations in *TSC1* (chromosome 9q34; hamartin) or *TSC2* (16p13; tuberin) (3, 4). Consistent with Knudson's "two-hit" tumor suppressor model (5), loss of heterozygosity (LOH) or a second inactivating mutation of *TSC2* is often detected (6). Inactivation of *TSC1* or *TSC2* leads to constitutive activation of mTOR (mechanistic target of rapamycin) and uncontrolled cell growth and proliferation. Thorough characterization of a lung LAM cell has been hampered by the lack of a clonal population of *TSC2*-null cells. LAM cells, as defined by *TSC2* mutation or LOH, do not grow in culture without wild-type cells and can only be detected after enrichment, adding complexity to the analysis of expression and genetic profiles of the LAM cell.

Although inactivating mutations of *TSC1* or *TSC2* are frequently found in LAM patient samples, *TSC1* or *TSC2* mutations or mTOR activation were not detected in some lung nodules (7), suggesting that alternative genetic changes may contribute to LAM pathogenesis. *TSC2* LOH can be detected in LAM lesions from many sources, including lung, kidney, and lymph nodes (6). Interestingly, smooth muscle, fat, and several vascular cell types from angiomyolipomas all have *TSC2* LOH (8). Circulating cells isolated from blood and other body fluids also contain *TSC2* LOH (9); however, *TSC2* LOH can be present in circulating cells isolated from patients with other lung diseases and cancers (10). Clearly, the genetics of LAM have not been completely elucidated.

The phenotype of a LAM cell is dependent on its source (11). The markers of a lung LAM cell are not necessarily the markers of an angiomyolipoma cell or a circulating cell, and a thorough examination across the cell types has not been done (11). The origin of the LAM cell remains elusive, and the LAM cell may retain stem cell characteristics or the potential to differentiate. These are critical issues—phenotypic and genetic characterization of the LAM cell and its recruited cells in the stroma, the origin of LAM cells, the lack of a cell model to elucidate cellular and molecular mechanisms, and why a predominantly female presentation—in understanding LAM pathogenesis.

Guo and colleagues (1) have taken great strides to shed light on three of the key issues. First, using single cell/nuclei RNA sequencing on LAM and normal lungs, they identified a unique cell type, the LAM^{CORE} cell, that expressed known LAM markers and was closely related to lung mesenchymal cells. Second, this cell type also had a gene expression pattern similar to that of uterine cells, suggesting a uterine origin for the LAM^{CORE} cell. Third, the gene expression pattern of the stromal cells of LAM lung suggested their recruitment and activation to further LAM pathogenesis.

Upon examining the RNA sequencing data from LAM lungs, 18 different cell types were identified, including the LAM^{CORE} cells that expressed previously identified LAM cell markers, for example, gp100, smooth muscle actin, estrogen receptor, and vascular endothelial growth factor D. The LAM^{CORE} signature genes suggested processes that are activated in LAM cells, including extracellular matrix organization, collagen and muscle fiber formation, cell migration and adhesion, prostaglandin-related processes, and development of vessels, neurons, and the urogenital system. Activated pathways included mTOR, PI3K–AKT, and ERK/MAPK, among others. Interestingly, the expression of proliferation markers was not increased in the LAM^{CORE} cells, confirming the idea of LAM as a "benign" metastatic neoplasm (2, 12).

The LAM lung nodule is composed of LAM cells with dysfunctional *TSC* genes and wild-type stromal cells, including type II pneumocytes, lymphatic endothelial cells, fibroblasts, lymphocytes, and mast cells (11, 13). It is presumed, as in other neoplasms, that the LAM cell recruits or influences wild-type cells in its microenvironment. To explore this concept, the expression of secretome proteins by the LAM^{CORE} cell was examined. The majority of LAM lung–specific secretome proteins were associated with tumorigenesis of the female reproductive tract and/or uterine carcinoma, consistent with idea that LAM cell metastasis originated from the uterus. In addition, changes in gene expression were detected in lung stromal cells, including lymphatic endothelial, airway and alveolar epithelial, and inflammatory cells. LAM-associated lymphatic endothelial cells were primed for metastatic invasion, with increased expression of proteins controlling lymphangiogenesis. Inflammatory cells may be recruited by the increased expression of cytokines and chemokines. The LAM^{CORE} cell is the director of its cast of cell types, which results in the range of histopathological changes seen in the LAM lung.

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It is important to plumb the depths of pathways implicated in LAM pathogenesis to determine druggable targets. The data on the LAM^{CORE} cell, its gene expression pattern, and its effect on its microenvironment were generated with the input of only three LAM lungs owing to the difficulty of obtaining fresh tissue samples. However, many of the pathways and processes proposed here fit with data already available about LAM pathogenesis. It is interesting to note that LAM^{CORE} cells could not be detected in the lung from a patient who had been taking sirolimus, the only approved drug for LAM that was tested in a double-blind clinical trial (14), suggesting that the LAM^{CORE} cell is sensitive to sirolimus and is indeed involved in disease pathogenesis. It would be interesting to see the expression pattern of the metastatic, circulating LAM cells and if they differ from the LAM^{CORE} cell. And although the theorized uterine origin of the LAM cell is promising and deserves further investigation, it does not explain the rare occurrence of LAM in males (15). LAM cells in the lung may arise from another site, which would, of necessity, be the case in males. Nevertheless, this study has given the LAM scientific community opportunities for future studies and represents a major advance in our understanding of this disease. ■

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Supporting a Precious Resource: Healthcare Clinicians

The well-being of frontline clinicians has received attention over the years (1). But the coronavirus disease (COVID-19) pandemic and its impact on clinicians smacked us all in the face with this reality—images of nurses with bruises on their faces from wearing personal protective equipment, stories of clinicians succumbing to suicide, and a seemingly never-ending surge of patients. Although evidence is building to show the impact of COVID-19 on clinicians, the essentialness of clinicians as one of the most, if not the greatest, precious resource in health care has never been clearer.

In this issue of the *Journal*, Azoulay and colleagues (pp. 1388–1398) examined symptoms of anxiety, depression, and peritraumatic dissociation in clinicians from 21 ICUs in France during spring 2020 (2). Nearly half of respondents reported anxiety, and a third reported depression and peritraumatic dissociation; these data are consistent with reports from other countries (3, 4). The sheer prevalence of anxiety, depression, and peritraumatic dissociation is staggering. The authors also identified six individual and organizational modifiable factors. Four factors associated with increased depression, anxiety, and disssociation were related to clinicians' emotions and circumstances. Fear was associated with increased odds of anxiety (odds ratio, 1.21; 95% confidence

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