Human Pathology (2016) **50**, 34–42

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Human PATHOLOGY

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Original contribution

Bronchial involvement in advanced stage lymphangioleiomyomatosis: histopathologic and molecular analyses☆,☆☆

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Received 26 July 2015; revised 27 October 2015; accepted 5 November 2015

Keywords:
Airway obstruction;
VEGF-D;
HIF-1α;
Lung transplantation;
Lymphangiogenesis;
Lymphangioleiomyomatosis;
mTORC1

Summary Lymphangioleiomyomatosis (LAM), a rare progressive disease that almost exclusively affects women, is characterized by pulmonary cysts and neoplastic proliferation of smooth muscle–like cells (LAM cells). Airflow obstruction is a physiologic consequence that is commonly observed in LAM and has been attributed to narrowing of peripheral airways. However, histopathologic examinations of the entire airway have been precluded by the limited availability of such specimens. Here, we used explanted lung tissues from 30 LAM patients for a thorough histologic analysis with a special emphasis on the bronchi. We found bronchial involvement by LAM cells and lymphatics in all patients examined. Furthermore, a moderate to severe degree of chronic inflammation (73%), goblet cell hyperplasia (97%), squamous cell metaplasia

☆ Competing interests: The authors declare no conflicts of interests.

☆☆ Funding/support: This study was supported by a Grant-in-Aid for Young Scientists (No. 24790363 to T.H.) and Scientific Research (No. 21659210 to K.S.) by the High Technology Research Center Grant from the Ministry of Education, Culture, Sports, Science, and Technology, Japan and, in part, by a grant to the Respiratory Failure Research Group from the Ministry of Health, Labour, and Welfare, Japan (K.S.).

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http://dx.doi.org/10.1016/j.humpath.2015.11.002
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(83%) of the epithelium, and thickening of basal lamina (93%) were identified in the bronchi. Because LAM cells are transformed by the functional loss of the *TSC* genes leading to a hyperactivated mammalian target of rapamycin complex 1 (mTORC1) signaling pathway, we confirmed the expression of phospho-p70S6K, phospho-S6, phospho–4E-BP1, and vascular endothelial growth factor (VEGF)-D in LAM cells from all of the patients examined. In contrast, no protein expression of hypoxia-inducible factor 1α, a downstream molecule indicative of mTORC1 activation and leading to VEGF production, was detected in any patient. Our study indicates that late-stage LAM patients commonly have bronchi involved by the proliferation of both LAM cells and lymphatics and that chronic inflammation complicated their disease. Furthermore, the up-regulation of hypoxia-inducible factor 1α, a common event in mTORC1-driven tumor cells, does not occur in LAM cells and plays no role in VEGF-D expression in LAM cells.
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1. Introduction

Lymphangioleiomyomatosis (LAM) is a rare systemic disorder usually found only in women and is characterized by a neoplastic proliferation of smooth muscle–like cells (LAM cells) and LAM-associated lymphangiogenesis. These LAM cells lead to cystic destruction of the lungs, chylous effusions, thoracic and abdominal lymphangioleiomyomas, and, frequently, renal angiomyolipoma and uterine LAM [1–3]. Clinically, the most common abnormality identified in pulmonary function tests of LAM patients is airflow obstruction. Standard pulmonary function and mechanics studies showed that expiratory flows were reduced predominantly because of airway narrowing or obstruction rather than loss of pulmonary elastic recoil force in LAM patients [4]. The involvement of bronchioles by proliferating LAM cells has already been reported by histopathologic studies [1,5]. The histopathology of bronchi, however, in LAM patients is not yet fully understood. The underlying problem is that the surgical specimens of LAM-affected lungs that are usually obtained for diagnosis by video-assisted thoracoscopic surgery do not include adequate bronchi for analysis.

Over the last few decades, remarkable progress has been made in the understanding of LAM cell biology. Accumulating evidence has shown that LAM is a low-grade, destructive, metastasizing neoplasm caused by a functional loss of *tuberous sclerosis complex* (*TSC*) genes and consequent hyperactivation of the mammalian target of the rapamycin complex 1 (mTORC1) signaling pathway [6]. Furthermore, vascular endothelial growth factor (VEGF)-D, a lymphangiogenic growth factor, appears to be an important participant in the lymphangiogenesis of LAM lesions [7,8]. The activation of mTORC1 in TSC-deficient cells results in the accumulation of hypoxia-inducible factor 1α (HIF-1α) and increases the expression of HIF-responsive genes including *VEGF in vitro* [9]. Thus, there is the possibility that the constitutive activation of mTORC1 signaling could lead to the activation of HIF-1α and consequent expression of VEGF-D in LAM cells. However, because no prior studies have addressed the potential activation of HIF-1α in LAM cells, that possibility is the focus of this investigation.

Here, we performed histopathologic analyses of explanted lungs from 30 patients at an advanced stage of pulmonary LAM and placed special emphasis on the histopathologic factors in the airways. The immunohistochemistry, Western blots, and reverse-transcription polymerase chain reaction (RT-PCR) analyses we used focused on mTORC1 signaling, HIF-1α, and VEGF-D expression in the LAM cells.

2. Materials and methods

2.1. Study population

The study population consisted of 30 women with pulmonary LAM who had undergone lung transplantation between 2003 and 2013 at Tohoku University Hospital, Kyoto University Hospital, Fukuoka University Hospital, or Okayama University Hospital (Table 1). The patient ages when the lung transplantations were performed ranged from 24 to 55 years (median, 42 years). Two patients (patients 6 and 16) met the diagnostic criteria for TSC (TSC-LAM) [10], but the others did not (sporadic LAM). The most common symptom was dyspnea (37%; 11 patients), followed closely by pneumothorax (33%; 10 patients), and the median duration of disease was 8 years with a range of 2 to 18 years. No patients had been treated with an mTOR inhibitor. Nineteen had never been smokers and 10 were ex-smokers, with an average of 5 packs/y. No patients had obvious occupational exposure to potentially pathogenic materials. This study was approved by the Ethics Committee of Juntendo University School of Medicine (2014013).

2.2. Histopathologic and immunohistochemical examinations of explanted lungs

Surgically resected lungs were fixed in 10% buffered formalin. The lung tissue samples were cut at 5-mm intervals along the lower bronchus, except for those from 7 patients (patients 1, 6, 7, 9, 10, 11, and 16), and embedded in paraffin after routine processing (median, 20 blocks per patient; ranging from 10 to 21 blocks). Each tissue block was sectioned at 4 μm and stained with hematoxylin-eosin (H&E) and Elastica-

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