Human Pathology (2015) **46**, 1685–1693

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Intracrine steroid production and mammalian target of rapamycin pathways in pulmonary lymphangioleiomyomatosis☆

Koko Adachi MD[a,b], Yasuhiro Miki DVM, PhD[a], Ryoko Saito MD, PhD[a], Shuko Hata PhD[a], Masanori Yamauchi MD, PhD[b], Yoshiki Mikami MD, PhD[c,1], Yoshinori Okada MD, PhD[d], Kuniaki Seyama MD, PhD[e], Takashi Kondo MD, PhD[d], Hironobu Sasano MD, PhD[a,*]

[a]*Department of Pathology, Tohoku University Graduate School of Medicine, Sendai 980-8575, Japan*
[b]*Department of Anesthesiology, Tohoku University Graduate School of Medicine, Sendai 980-8575, Japan*
[c]*Department of Diagnostic Pathology, Kyoto University Hospital, Kyoto 606-8507, Japan*
[d]*Department of Thoracic Surgery, Institute of Development, Aging and Cancer, Tohoku University, Sendai 980-8575, Japan*
[e]*Department of Respiratory Medicine, Juntendo University Faculty of Medicine, Tokyo 113-8431, Japan*

Received 22 September 2014; revised 14 February 2015; accepted 21 February 2015

Keywords:
Aromatase;
Bcl-2;
Estrogen;
mTOR;
Pulmonary lymphangioleiomyomatosis

Summary Lymphangioleiomyomatosis (LAM) is a rare, potentially fatal disease primarily affecting young women. Estrogens enhance cell proliferation and progression of the tumor. Clinical trials using molecularly targeted agents such as endocrine manipulation and mammalian target of rapamycin (mTOR) inhibitors are in progress, but the status of these molecules, including aromatase and mTOR, has not been explored in LAM tissue. We first examined immunoreactivity for sex steroid receptors (estrogen receptor [ER] α, ERβ, progesterone receptor, androgen receptor), sex steroid–synthesizing enzymes (aromatase, steroid sulfatase, 17β-hydroxysteroid dehydrogenase 1, 5α-reductases), apoptotic suppression factor (Bcl-2), and factors involved in the mTOR signaling pathway in 30 pulmonary LAM tissues. Immunoreactivity for ERα, ERβ, progesterone receptor, aromatase, and Bcl-2 was significantly more abundant in epithelioid cells, whereas the status of androgen receptor, 5α-reductases, and phospho-mTOR signaling was not different in epithelioid and spindle-shaped LAM cells. We further examined the correlation among *H* scores of these markers using hierarchical clustering analysis. The results indicated that LAM tumors can be further classified into "aromatase" and "mTOR" groups on the basis of the patterns of immunoreactivity, and the 2 types could benefit from different modes of therapy.

1. Introduction

Lymphangioleiomyomatosis (LAM) is a rare progressive and occasionally fatal pulmonary disease associated with abnormal proliferation of smooth muscle cells, also termed *LAM cells* [1]. Histologically, the following 2 types of LAM

[1] Current address: Department of Surgical Pathology, Kumamoto University Hospital, Kumamoto 860-0811, Japan.

☆ Competing interest: There are no conflicts of interest for any of the authors.

* Corresponding author at: Department of Pathology, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8575 Japan.
E-mail address: hsasano@patholo2.med.tohoku.ac.jp (H. Sasano).

http://dx.doi.org/10.1016/j.humpath.2015.02.019
0046-8177/

cells can be identified (Fig. 1): (1) small, spindle-shaped cells with higher proliferative activity, usually located centrally in the LAM nodules, and (2) large epithelioid cells distributed mainly along the periphery of the nodules and usually immunopositive for estrogen receptors (ERs) and progesterone receptors (PRs) [2,3]. Among the factors studied so far, Bcl-2 was reported to be involved in homeostasis of LAM, and the BCL2 proto-oncogene is considered an ER target gene in LAM cells [4].

A LAM tumor is characterized by its usual occurrence in premenopausal female patients and being markedly exacerbated by pregnancy [1,5,6]. Therefore, LAM is considered one of the sex steroid–dependent diseases. However, there have been no randomized placebo-controlled trials confirming the efficacy of hormone therapy, and the European Respiratory Society criteria by no means recommend the use of hormonal manipulation as a routine treatment [3,7,8]. It is also true that studies focusing on the potential roles of estrogens in the progression of LAM indicated that antiestrogen therapy, including aromatase inhibitors, could be effective in the control of LAM [9–11]. However, the status and potential roles of intracrine estrogen-producing enzymes such as aromatase, 17β-hydroxysteroid dehydrogenase type 1 (17βHSD1), and steroid sulfatase (STS), as well as the enzymes involved in intracrine androgen production, including 5α-reductase types 1 and 2, all of which play important roles in human breast cancer, have not been examined at all in LAM patients.

The mutational inactivation of the tumor suppressor complex genes tuberous sclerosis complex (*TSC*) 1 and *TSC2* has been detected in lung LAM cells [6,12–14]. The *TSC* genes function as negative regulators of mammalian target of rapamycin (mTOR), a major controller of cell growth, metabolism, and survival, and rapamycin analogs have recently been used to treat LAM patients, with promising results [10,15,16]. The mTOR inhibitor sirolimus [17] was associated with a reduction of angiomyolipoma in patients with TSC and sporadic LAM [15,16]. However, the status of the mTOR pathway in LAM tissue has not been explored at all.

In this study, we first examined the status of aromatase, 17βHSD1, STS, and androgen-related factors; androgen receptor (AR) and 5α-reductases; and ER and PR in 30 LAM cases using immunohistochemistry. We then evaluated the status of activated (phosphorylated [p-]) mTOR, including downstream ribosomal protein S6 kinase (p-S6K) and the eukaryotic translation initiation factor 4E-binding protein (p-4E-BP1), in 20 LAM tissues.

2. Materials and methods

2.1. LAM patients

Tumor specimens were obtained by surgical excision of the lung in the Department of Surgery, Tohoku University Hospital, Sendai, and Kyoto University Hospital, Kyoto, Japan. Specimens for immunohistochemistry examination were fixed in 10% formalin and embedded in paraffin wax. Informed consent was obtained from all the patients prior to their surgery. Research protocols for this study were approved by the Ethics Committees at both Tohoku and Kyoto University School of Medicine. Hormone-untreated and premenopausal patients could have high concentrations of estrogen in the blood. On the other hand, treated and postmenopausal patients could have low concentrations of estrogen in the circulation. Therefore, these patients were classified into 2 groups: those without hormone therapy and premenopausal status and those receiving hormone therapy or postmenopausal. The first group of the patients was tentatively termed the *estrogen-secretion group* and the second the *non–estrogen-secretion group*.

The thirty patients were all women. Clinicopathological findings are summarized in Table 1. The average age of the patients was 40.7 ± 10.5 years. Postmenopausal was tentatively defined as more than 50 years. Among these 30 patients,

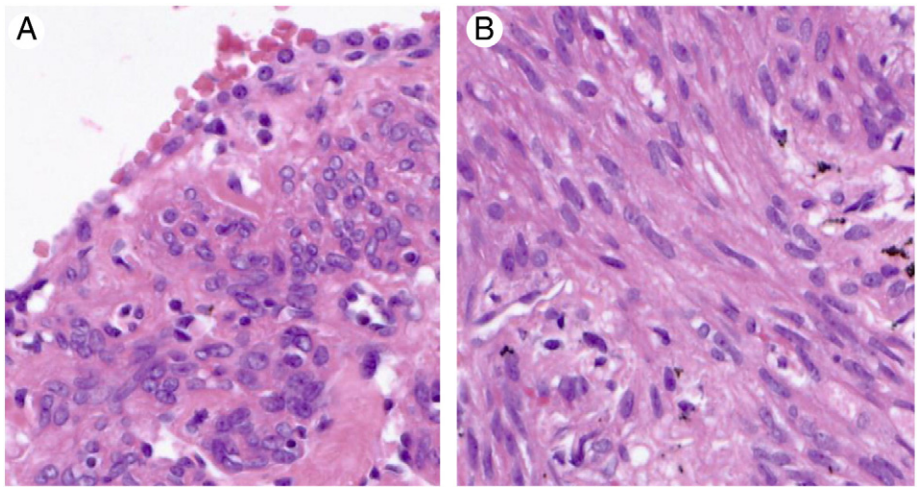

Fig. 1 Hematoxylin and eosin staining of LAM tissues. A, Epithelioid cells. B, Spindle-shaped cells. Original magnification ×400.