Human Pathology (2018) **79**, 199–207

ELSEVIER

Human PATHOLOGY

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Original contribution

In situ analysis of mTORC1/2 and cellular metabolism–related proteins in human Lymphangioleiomyomatosis☆,☆☆

Ildiko Krencz MD[a,1], Anna Sebestyen MSc, PhD[a,1], Judit Papay MD, PhD[a], Andras Jeney MD, PhD, DSc[a], Zoltan Hujber PharmD[a], Charles D. Burger MDFCCP[b], Cesar A. Keller MD[b,c], Andras Khoor MD, PhD[d,*]

[a]*1st Department of Pathology and Experimental Cancer Research, Semmelweis University, H-1085 Budapest, Hungary*
[b]*Division of Pulmonary, Allergy and Sleep Medicine, Mayo Clinic, Jacksonville, FL 32224, United States*
[c]*Division of Transplant Medicine, Mayo Clinic, Jacksonville, FL 32224, United States*
[d]*Department of Laboratory Medicine & Pathology, Mayo Clinic, Jacksonville, FL 32224, United States*

Received 4 March 2018; revised 11 May 2018; accepted 24 May 2018

Keywords:
Lymphangioleiomyomatosis;
mTORC1;
mTORC2;
Cellular metabolism;
Immunohistochemistry

Summary Lymphangioleiomyomatosis (LAM) is a rare progressive cystic lung disease with features of a low-grade neoplasm. It is primarily caused by mutations in *TSC1* or *TSC2* genes. Sirolimus, an inhibitor of mTOR complex 1 (mTORC1), slows down disease progression in some, but not all patients. Hitherto, other potential therapeutic targets such as mTOR complex 2 (mTORC2) and various metabolic pathways have not been investigated in human LAM tissues. The aim of this study was to assess activities of mTORC1, mTORC2 and various metabolic pathways in human LAM tissues through analysis of protein expression. Immunohistochemical analysis of p-S6 (mTORC1 downstream protein), Rictor (mTORC2 scaffold protein) as well as GLUT1, GAPDH, ATPB, GLS, MCT1, ACSS2 and CPT1A (metabolic pathway markers) were performed on lung tissue from 11 patients with sporadic LAM. Immunoreactivity was assessed in LAM cells with bronchial smooth muscle cells as controls. Expression of p-S6, Rictor, GAPDH, GLS, MCT1, ACSS2 and CPT1A was significantly higher in LAM cells than in bronchial smooth muscle cells ($P < .01$). No significant differences were found between LAM cells and normal bronchial smooth muscle cells in GLUT1 and ATPB expression. The results are uniquely derived from human tissue and indicate that, in addition to mTORC1, mTORC2 may also play an important role in the pathobiology of LAM.

Abbreviations: ACSS2, acyl-coenzyme A synthetase short-chain family member 2; ATPB, β-F1-ATPase; BSM, bronchial smooth muscle; CPT1A, carnitine palmitoyltransferase 1A; ER, estrogen receptor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GLS, glutaminase; GLUT1, glucose transporter 1; HMB-45, homatropine methylbromide-45; LAM, lymphangioleiomyomatosis; MCT1, monocarboxylate transporter 1; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; p-S6, phosphorylated ribosomal S6 protein; Rictor, rapamycin-insensitive companion of mammalian target of rapamycin; SMA, smooth muscle actin; TSC2, tuberous sclerosis 2 (tuberin).

☆ Disclosures/Conflict of Interest: none.

☆☆ Funding/Support: This work was supported by the ÚNKP-17-3 New National Excellence Program of The Ministry of Human Capacities (I. K.), scientific grant of the Hungarian Respiratory Foundation (I. K.), Bolyai Fellowship of Hungarian Academy of Sciences (A. S.), and Semmelweis University Innovation Found STIA-KF-17 (A. S.).

* Corresponding author at: Department of Laboratory Medicine & Pathology, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224.
E-mail address: khoor.andras@mayo.edu (A. Khoor).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.humpath.2018.05.018

Furthermore, glutaminolysis, acetate utilization and fatty acid β-oxidation appear to be the preferred bioenergetic pathways in LAM cells. mTORC2 and these preferred bioenergetic pathways appear worthy of further study as they may represent possible therapeutic targets in the treatment of LAM.

1. Introduction

Lymphangioleiomyomatosis (LAM) is a rare multisystem disorder with a strong female predilection, manifesting primarily as a progressive, diffuse cystic lung disease. Symptoms are generally due to obstructive lung disease; however, pleural complications such as pneumothorax and chylothorax may also occur. The lung cysts are related to proliferation of immature smooth muscle cells of perivascular phenotype (LAM cells) [1] and LAM is now considered as a low-grade neoplasm of the perivascular epithelioid cell tumor family [2]. The term sporadic LAM is used for patients without the tuberous sclerosis complex (TSC), while TSC-LAM refers to LAM that occurs in the setting of TSC. Both forms are primarily caused by *TSC1* or *TSC2* gene mutations [3]. These mutations lead to hyperactivation of the mammalian target of rapamycin (mTOR) and subsequently proliferation of LAM cells [4].

mTOR is a component of two multiprotein complexes: mTOR complex 1 and mTOR complex 2 (mTORC1 and mTORC2) (Fig. 1). In addition to mTOR, mTORC1 contains scaffold protein Raptor and mTORC2 contains scaffold protein Rictor [5]. Through its participation in mTORC1 and mTORC2, mTOR integrates a variety of environmental signals and regulates cell growth and homeostasis [6]. Activation of mTORC1 leads to phosphorylation of eukaryotic initiation factor 4E-binding protein 1 (4EBP1), S6 kinase (S6K), and ribosomal S6 protein. Phospho-S6 (p-S6) facilitates protein translation, cell growth, and proliferation. mTORC1 also influences cellular metabolism (Fig. 1). Alternatively, mTORC2 is a mediator of actin cytoskeletal organization and promotes cell survival via phosphorylation of protein kinase B and serum- and glucocorticoid-induced protein kinase [5, 6].

mTOR also plays a central role in metabolic reprogramming of neoplastic cells with altered utilization of glucose, glutamine, and lipids [5-9]. Increased glucose uptake and overexpression of glucose transporter 1 (GLUT1) is well documented in most neoplasms [10, 11]. Regardless, certain neoplasms, including LAM, are undetectable by positron emission tomography using 2-deoxy-2-[18F]fluoro-D-glucose [12-14], suggesting that these neoplasms may use an alternative energy source such as glutamine or acetate instead of glucose.

Sirolimus is an mTORC1 inhibitor, which has been successfully utilized to attenuate disease progression in LAM patients [15, 16]. Unfortunately, lost lung function is not restored and disease progression resumes once treatment is discontinued [16]. In addition, certain disease subgroups, such as those that are post-menopausal, may have limited if any benefit from sirolimus. Lastly, the most effective dose and treatment duration are unknown, although presumed to be lifelong. Of course, some patients are unable to tolerate the treatment due to adverse events [17, 18]. It is therefore important to identify pathobiological targets that may respond to new or additional therapeutics to mitigate the proliferation of LAM cells.

The purpose of this study was to assess the significance of mTORC1, mTORC2 and various metabolic pathways (including glycolysis, oxidative phosphorylation, glutaminolysis, fatty acid β-oxidation, and acetate utilization) in the pathogenesis of LAM, using semiquantitative immunohistochemical methods on formalin-fixed paraffin-embedded human lung tissue.

2. Materials and methods

2.1. Tissues

Our study was approved by the Mayo Clinic Institutional Review Board. Formalin-fixed paraffin-embedded lung tissue was available from the lung tissue registry for 11 patients with sporadic LAM. These patients underwent lung transplantation (7 patients) and diagnostic wedge biopsies (4 patients) at Mayo Clinic in Jacksonville, Florida, between January 1, 2004 and December 31, 2016. Diagnosis of LAM was based on the presence of characteristic clinical, radiologic, and histologic findings and was confirmed by immunoreactivity for smooth muscle actin (SMA), HMB-45 and β-catenin. Clinical and pathologic parameters are summarized in Table 1. Two patients (patient no. 6 and 8) received hormone therapy and none of the patients received mTOR inhibitor therapy prior to surgery.

2.2. Immunohistochemistry

Representative tissue blocks were selected for each patient by a pulmonary pathologist (AK). Immunohistochemistry was performed on 4 μm-thick sections of formalin-fixed paraffin-embedded tissue. After deparaffinization and endogenous peroxidase blocking, antigen retrieval was performed for 20 to 30 minutes (buffers: 10 mM citrate pH 6.0; 10 mM Tris-EDTA pH 9.0; cell conditioning 1 antigen retrieval buffer pH 8.5 [Ventana Medical Systems, Inc.]; Target Retrieval Solution buffer pH 9.0 [Dako]). Slides were incubated with the diluted primary antibodies summarized in Table 2. mTORC1 activity

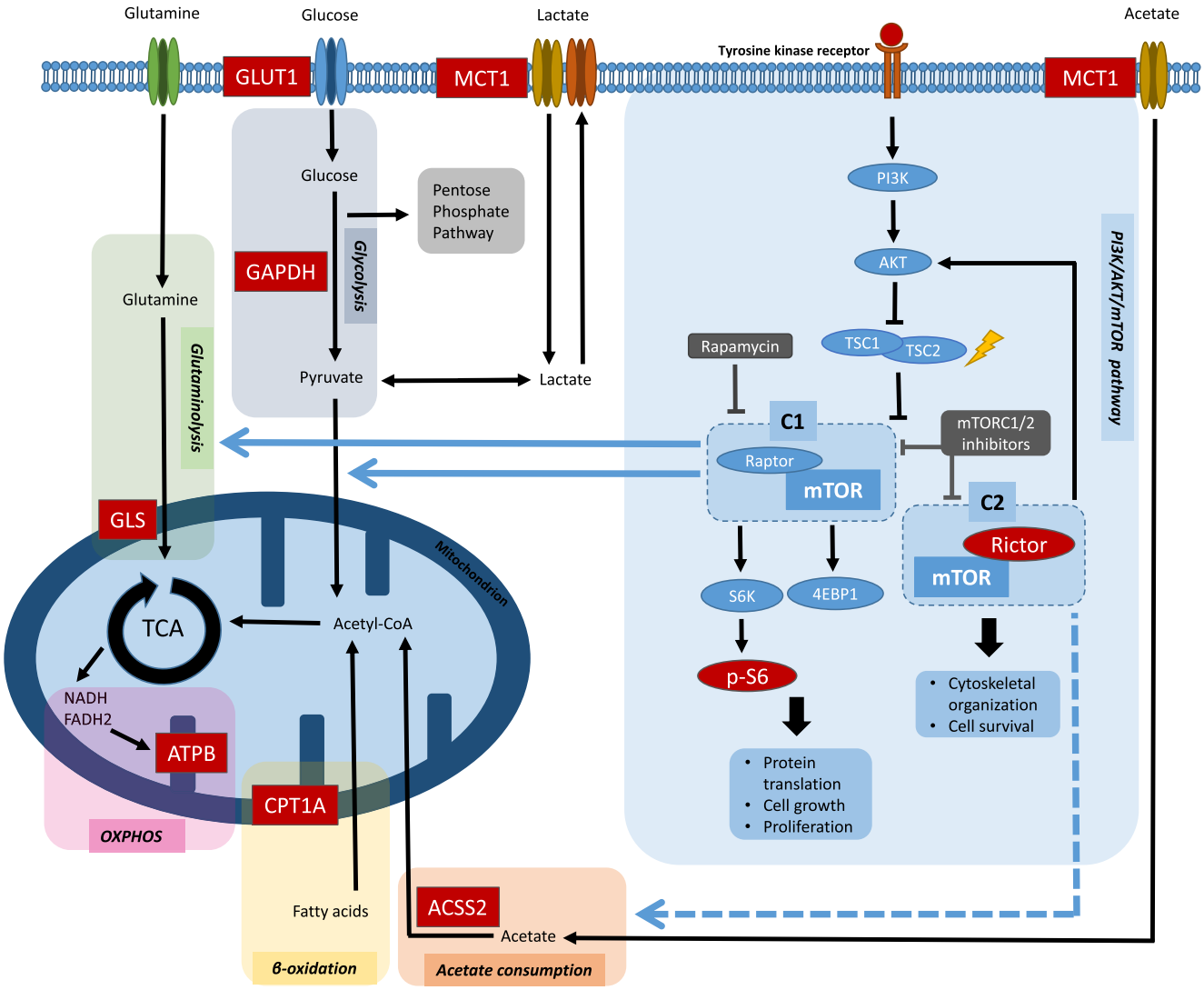

Fig. 1 Simplified scheme of studied PI3K/Akt/mTOR and bioenergetic pathways.Proteins analyzed by immunohistochemistry are shown in red. LAM cells have an aberrant activation of mTOR caused by mutations in *TSC1* or *TSC2* genes. mTOR protein kinase is present in 2 multiprotein complexes, mTORC1 and mTORC2, which are characterized by different scaffold proteins, Raptor and Rictor, respectively. Activation of mTORC1 enhances protein translation and cell growth and proliferation via phosphorylation of eukaryotic initiation factor 4E-binding protein and activation S6 kinase (S6K). Activation of S6K leads to phosphorylation of S6 ribosomal protein. In contrast, mTORC2 is a mediator of actin cytoskeletal organization and promotes cell survival. mTOR complexes have a central role in metabolic regulation, including utilization of glucose, glutamine, acetate, and lipids. According to recent studies [8,32], activation of mTORC1 can stimulate glycolysis and glutaminolysis (solid blue lines). In LAM cells, a correlation between mTORC1 activity and glutaminolysis was detected. A strong positive correlation between expression of Rictor and ACSS2 was also detected in our study, which suggests that mTORC2 may influence utilization of acetate (dashed blue line).

was evaluated by expression of the downstream protein phospho-S6 (p-S6). In the absence of a strong antibody for a downstream protein, mTORC2 activity was estimated by expression of mTORC2 associated protein Rictor. Glycolysis and oxidative phosphorylation were characterized by GLUT1, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and β-F1-ATPase (ATPB), glutaminolysis by glutaminase (GLS), fatty acid β-oxidation by carnitine palmitoyltransferase 1A (CPT1A), and acetate utilization by acyl-coenzyme A synthetase short-chain family member 2 (ACSS2) and monocarboxylate transporter 1 (MCT1). Expression of LAM cell markers SMA, HMB-45 and β-catenin) and hormone receptors (estrogen and progesterone) was also examined. Immunohistochemical reactions were visualized by Novolink Polymer (Leica Biosystems) and VECTASTAIN Universal (Vector Laboratories) detection systems, using 3,3′-diaminobenzidine as chromogen (Fig. 2).

Immunostained sections were reviewed by 2 pathologists (I.K. and J.P.) with a focus on immunoreactivity for lesional LAM cells. As a minimum, three LAM nodules containing at least 100 cells per nodule were examined in each case. There is no proven precursor for the LAM cell; therefore, we used

Table 1 Clinicopathologic characteristics of LAM patients

Patient	Age (years)	Dx-Bx time (years)	Procedure	Lymph node metastasis	Smoking history	VEGF-D (pg/mL)	Treatment before procedure	
							Hormone therapy	mTOR inhibitor
1	59	0	Wedge biopsy	Unknown	Never	Not done	No	No
2	39	1	BLT	Yes	Former smoker	Not done	No	No
3	41	1	BLT	No	Former smoker	Not done	No	No
4	35	5	BLT	No	Never	Not done	No	No
5	37	5	BLT	Yes	Former smoker	Not done	No	No
6	39	3	BLT	Yes	Never	Not done	Yes	No
7	58	2	BLT	Yes	Never	Not done	No	No
8	49	11	BLT	No	Former smoker	Not done	Yes	No
9	34	0	Excision of bleb	Unknown	Never	Not done	No	No
10	43	0	Wedge biopsy	Unknown	Never	385	No	No
11	32	0	Wedge biopsy	Unknown	Never	8888	No	No

Abbreviations: BLT, bilateral lung transplantation; Bx, biopsy; Dx, diagnosis, mTOR, mammalian target of rapamycin; VEGF-D, vascular endothelial growth factor D.

bronchial smooth muscle (BSM) cells as an internal control. Comparison to BSM is consistent with the smooth muscle hypothesis of LAM cell derivation and the fact that both BSM cells and LAM cells are strongly positive for SMA (see Fig. 2.). Immunohistochemical expression of mTOR and metabolic markers was also analyzed in vascular smooth muscle cells in

Table 2 Primary antibodies and their conditions used in this study

Antibody	Antigen	Clone	Dilution	Antigen retrieval	Cellular localization	Significance	Supplier
LAM markers	β-Catenin	17C2	1:100	Tris-EDTA (pH 9)	Membranous and cytoplasmic	LAM marker	Novocastra Laboratories Ltd
	HMB-45	HMB-45	Predilute	TRS (pH 9)	Cytoplasmic	LAM marker	Dako
	SMA	1A4	1:400	Citrate (pH 6)	Cytoplasmic	LAM marker	Dako
Hormone receptors	ER	SP1	Predilute	CC1 (pH 8.5)	Nuclear	ER status	Ventana Medical Systems, Inc
	PR	1E2	Predilute	CC1 (pH 8.5)	Nuclear	PR status	Ventana Medical Systems, Inc
mTOR markers	p-S6	#2211	1:100	Citrate (pH 6)	Cytoplasmic	mTORC1	Cell Signaling Technology, Inc
	Rictor	A500-002A	1:1000	Citrate (pH 6)	Cytoplasmic	mTORC2	Bethyl Laboratories, Inc
Metabolic markers	GLUT1	ab652	1:400	Citrate (pH 6)	Membranous and cytoplasmic	Glucose uptake	Abcam
	GAPDH	ab8245	1:600	Citrate (pH 6)	Cytoplasmic and nuclear	Glycolysis	Abcam
	ATPB	ab14730	1:100	Citrate (pH 6)	Cytoplasmic	Oxidative phosphorylation	Abcam
	GLS	ab156876	1:200	Citrate (pH 6)	Cytoplasmic	Glutaminolysis	Abcam
	MCT1	A304-358A	1:100	Citrate (pH 6)	Membranous and cytoplasmic	Lactate and acetate uptake	Bethyl Laboratories, Inc
	ACSS2	#3658	1:200	Citrate (pH 6)	Cytoplasmic and nuclear	Acetate consumption	Cell Signaling Technology, Inc
	CPT1A	ab128568	1:500	Citrate (pH 6)	Cytoplasmic	β-oxidation of fatty acids	Abcam

Abbreviations: ACSS2, acyl-coenzyme A synthetase short-chain family member 2; ATPB, β-F1-ATPase; CC1, cell conditioning 1; CPT1A, carnitine palmitoyltransferase 1A; ER, estrogen receptor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GLS, glutaminase; GLUT1, glucose transporter 1; HMB-45, homatropine methylbromide 45; LAM, lymphangioleiomyomatosis; MCT1, monocarboxylate transporter 1; mTOR, mammalian target of rapamycin; mTORC, mammalian target of rapamycin complex; PR, progesterone receptor; p-S6, phosphorylated ribosomal S6 protein; Rictor, rapamycin-insensitive companion of mammalian target of rapamycin; SMA, smooth muscle actin.

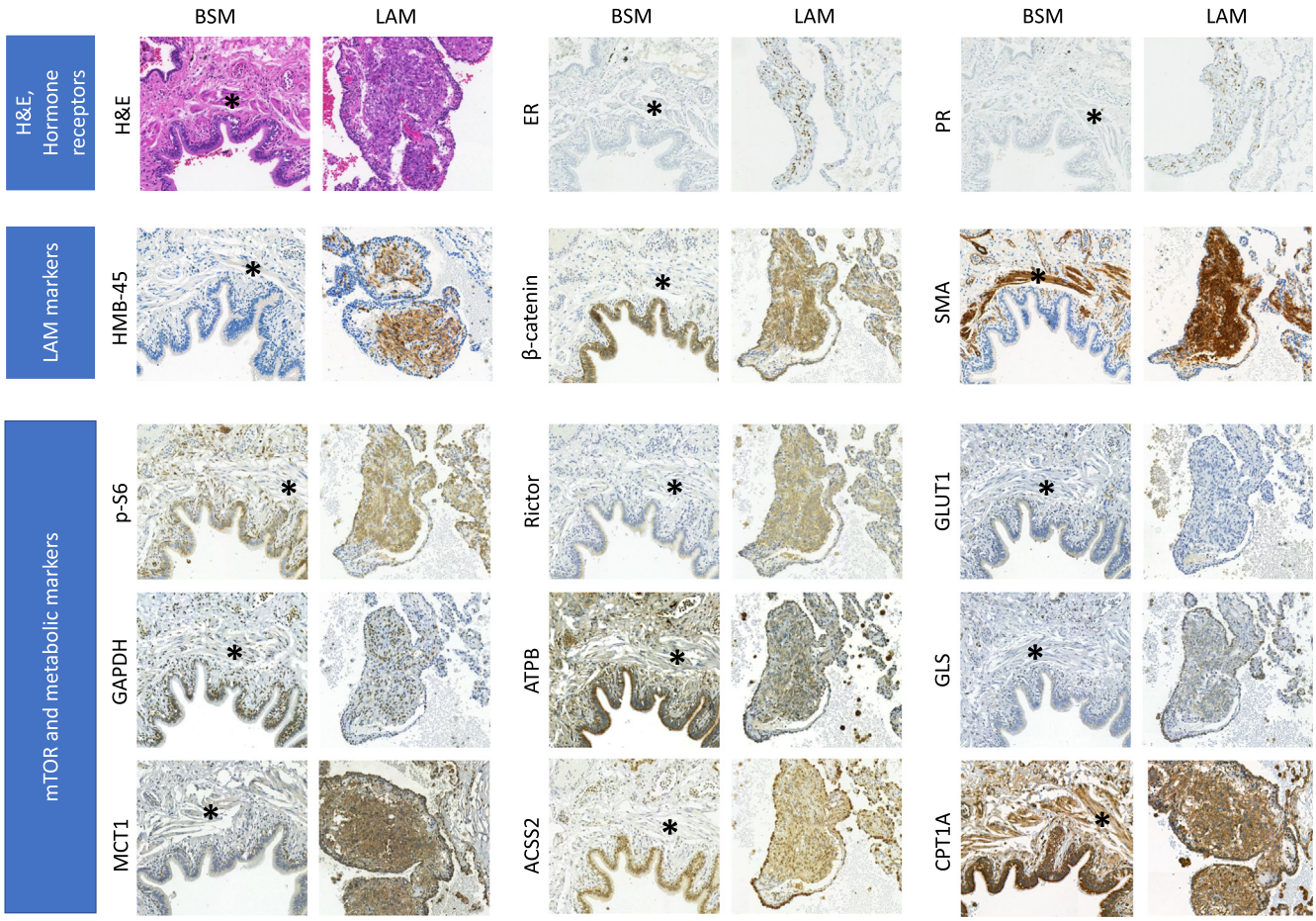

Fig. 2 Immunohistochemical stainings in LAM and BSM cells (patient no. 2) for LAM markers (β-catenin, HMB-45, SMA), hormone receptors (ER, PR), mTORC1/C2 markers (p-S6, Rictor), and metabolic markers (GLUT1, GAPDH, ATPB, GLS, MCT1, ACSS2, CPT1A).Original magnification ×200. BSM cells are indicated with asterisks (*).

each case. The expression in vascular smooth muscle cells was identical to that in BSM cells (data not shown). For mTOR and metabolic markers, an H-score was calculated by multiplying the fraction of immunopositive LAM cells (%) by staining intensity (0, 1+, 2+ or 3+), as previously described [19]. For example, 50% of LAM cells staining positive with 3+ intensity results in an H-score of 150. Based on the H-score, immunohistochemical expression of these markers was categorized as follows: no expression (H-score ≤ 10), low expression (H-score 11–100), and high expression (H-score> 100). For SMA, HMB-45 and β-catenin, any staining was considered positive. For estrogen and progesterone receptors, the percentage of positive cells was recorded.

2.3. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics software, version 22 (SPSS Inc.). The Wilcoxon signed rank test was used to evaluate the differences between LAM cells and matched normal BSM cells. The Mann–Whitney test and Spearman rank correlation were used to compare protein expressions and clinicopathologic parameters. A 2-tailed test was used to calculate statistical significance. To achieve highly reliable results, significance was defined as $P \leq .01$.

3. Results

3.1. Expression of LAM cell markers

Expression of LAM cell markers and hormone receptors are shown in Table 3. LAM cells were positive for HMB-45 in 9 of 11 cases (82%). All cases were positive for SMA and β-catenin.

3.2. Expression of mTOR-related proteins

In LAM cells, high p-S6 expression suggesting high mTORC1 activity was observed in 10 of 11 cases (91%), and high Rictor expression suggesting high mTORC2 activity

Table 3 Expressions of LAM markers (HMB-45, β-catenin, SMA) and hormone receptors (ER, PR) in LAM cells

Patient	HMB-45	β-Catenin	SMA	ER (%)	PR (%)
1	Positive	Positive	Positive	50	10
2	Positive	Positive	Positive	60	40
3	Negative	Positive	Positive	0	60
4	Positive	Positive	Positive	50	20
5	Positive	Positive	Positive	40	70
6	Positive	Positive	Positive	50	30
7	Positive	Positive	Positive	60	30
8	Positive	Positive	Positive	70	20
9	Positive	Positive	Positive	70	60
10	Negative	Positive	Positive	–	–
11	Positive	Positive	Positive	75	60

Abbreviations: ER, estrogen receptor; HMB-45, homatropine methylbromide 45; LAM, lymphangioleiomyomatosis; PR, progesterone receptor; SMA, smooth muscle actin.

was observed in 6 of 11 cases (55%) (Fig. 3). Low expression for both p-S6 and Rictor was observed in only 1 case. In contrast, no or low expression was observed in BSM cells for both of these markers in all cases. The difference between LAM and BSM cells was statistically significant for both p-S6 and Rictor ($P < .01$) (Fig. 4).

3.3. Expression of metabolic pathway–related proteins

3.3.1. Glycolysis and oxidative phosphorylation

Membranous or cytoplasmic immunoreactivity for GLUT1 (marker for glucose uptake) was detectable in LAM cells in 5 of 11 cases (45%), the expression was low in 4 cases and high in 1 (Fig. 3). Minimal or no GLUT1 expression was observed in normal BSM cells in all cases; however, the difference in H-scores between LAM and BSM cells was not statistically significant ($P = .61$) (Fig. 4).

Cytoplasmic immunoreactivity was evaluated for GAPDH (marker for glycolysis) and ATPB (marker for oxidative phosphorylation). In LAM cells, high expression of cytoplasmic GAPDH was detected in 7 cases (64%) and high expression of ATPB was detected in 4 (36%) (Fig. 3). On the other hand, BSM cells showed high expression of ATPB in only 1 case (9%) and high expression of GAPDH in none. The difference in H-scores between LAM and BSM cells was statistically significant for GAPDH ($P < .01$), but not for ATPB ($P = .02$) (Fig. 4).

3.3.2. Glutaminolysis

GLS (marker of glutaminolysis) was expressed in LAM cells in 10 of 11 cases (91%); in 7 cases (64%) the expression was high (Fig. 3). On the other hand, GLS expression was either low (1 case [9%]) or absent (10 cases [91%]) in BSM cells. The H-score for GLS was significantly higher in LAM cells compared to BSM cells ($P < .01$) (Fig. 4).

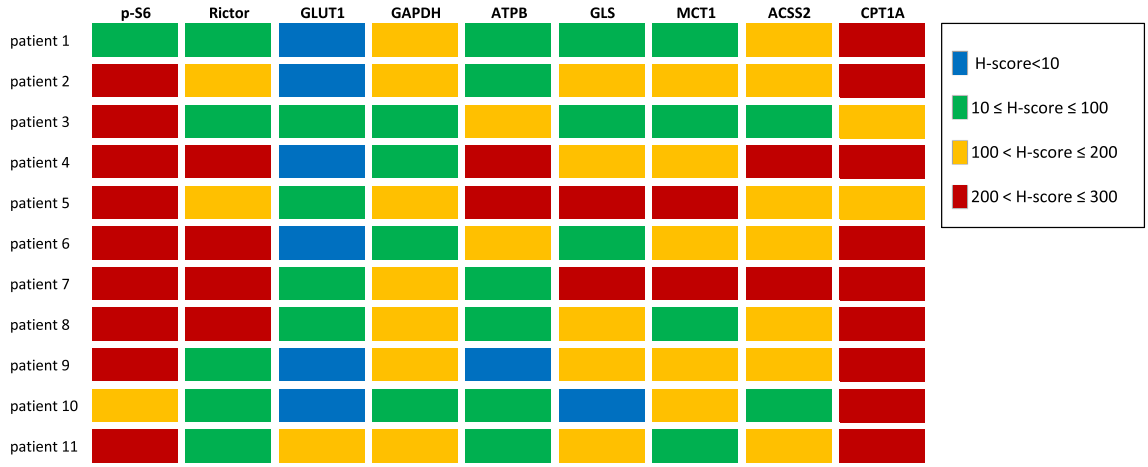

Fig. 3 H-scores for p-S6, Rictor, GLUT1, GAPDH, ATPB, GLS, MCT1, ACSS2, and CPT1A in all patients in LAM Cells.Immunohistochemical expression was categorized as follows: no expression (H-score ≤ 10), low expression (H-score 11–100), and high expression (H-score> 100).

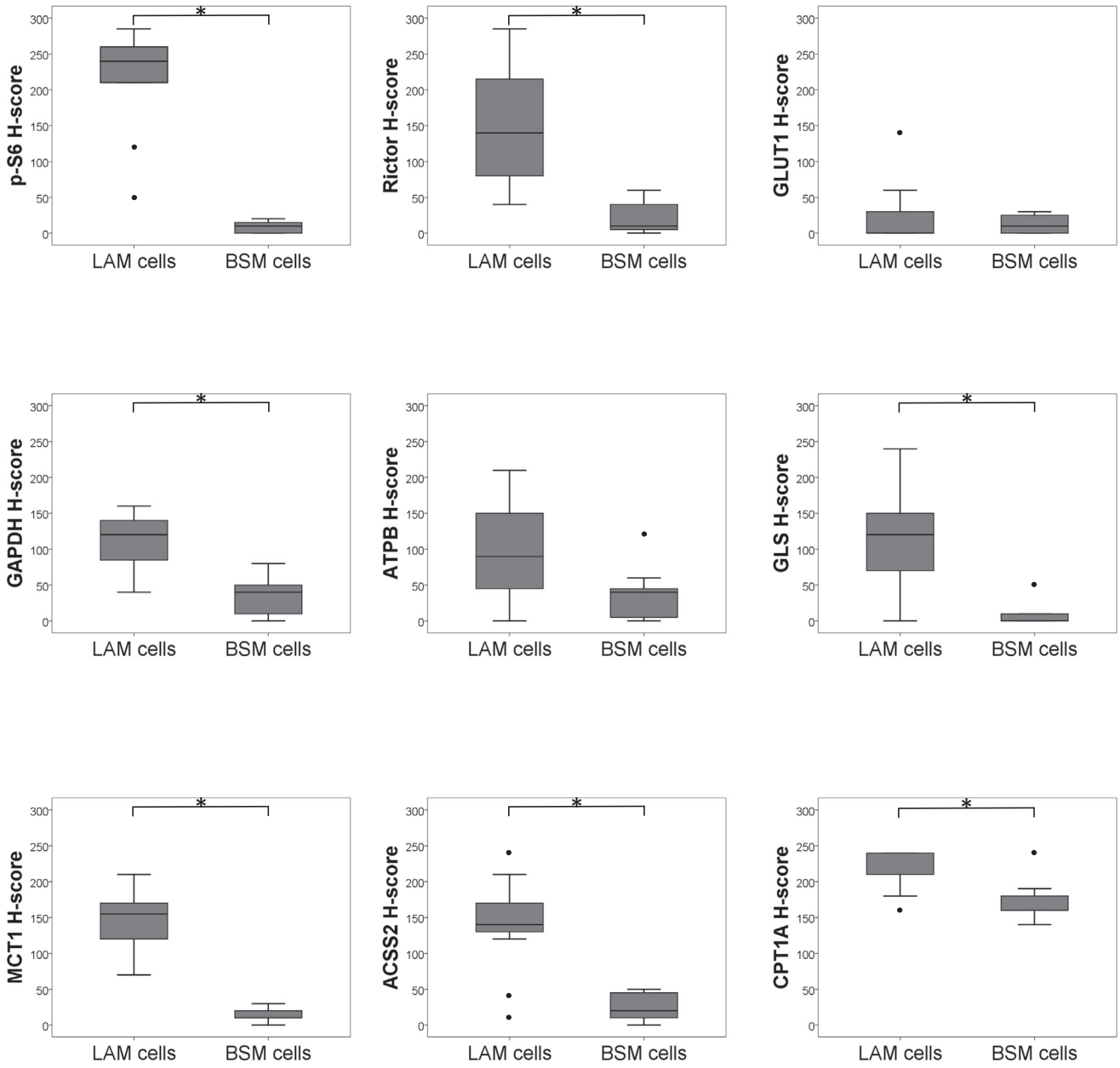

Fig. 4 Expression levels of mTOR and metabolic markers in LAM cells and BSM cells.H-scores of p-S6, Rictor, GAPDH, GLS, MCT1, ACSS2, and CPT1A were significantly higher in LAM cells than in BSM cells. In contrast, H-scores of GLUT1 and ATPB were not statistically different between LAM and BSM cells. *P ≤ .01 calculated with Wilcoxon signed rank test.

3.3.3. Fatty acid β-oxidation

Expression of CPT1A (rate-limiting enzyme in fatty acid β-oxidation) was high in both LAM and BSM cells in all cases (Fig. 3). Nevertheless, the mean H-score for CPT1A was significantly higher in LAM cells than in BSM cells (P < .01) (Fig. 4).

3.3.4. Acetate uptake and utilization

Expressions of MCT1 (transporter for monocarboxylates, eg, lactate and acetate) and ACSS2 (marker for the possibility of cellular acetate utilization) were high in LAM cells in 7 (64%) and 9 (82%) cases, respectively (Fig. 3). On the other hand, MCT1 and ACSS2 expressions were low or absent in BSM cells in all cases. The H-score was significantly higher in LAM cells than in BSM cells for both MCT1 and ACSS2 (P < .01) (Fig. 4).

3.4. Correlation between mTOR and metabolic pathway–related proteins

The association between expression of p-S6 and Rictor and that of metabolic pathway–related proteins was also studied. In LAM cells, positive correlation was detected between p-S6 and GLS (r = 0.732, P = .01) and between Rictor and

ACSS2 (r = 0.849, P < .01). In addition, positive correlation was observed between Rictor and ACSS2 in BSM cells (r = 0.769, $P < .01$).

3.5. Correlation between protein expression and clinicopathologic parameters

The H-score for both Rictor and ATPB appeared to be higher in explanted (end-stage) lungs than diagnostic wedge biopsies, but results were not statistically significant at P ≤ .01. Positive correlation was also observed between H-scores for CPT1A and estrogen receptor (ER) positivity (r = 0.808, $P < .01$). No other association was observed between protein expression and clinicopathologic parameters.

4. Discussion

Histologically, LAM is characterized by cystic spaces surrounded by bundles of proliferating LAM-type smooth muscle cells [20]. These LAM cells are typically immunoreactive for HMB-45, β-catenin, SMA, desmin, ER, and progesterone receptor [21-24].

mTOR plays an important role in the regulation of protein translation, cell growth, proliferation, cytoskeletal organization, and cellular metabolism [5, 8, 9] (Fig. 1). In this study, expression of p-S6 (downstream target of mTORC1) and Rictor (part of mTORC2) was used to estimate mTORC1 and mTORC2 activity, respectively. Findings suggested high mTORC1 and mTORC2 activity in LAM cells in 91% and 55% of the cases, respectively. High mTORC1 activity in LAM cells is consistent with previous observations [19, 25]. Our findings suggest that high mTORC2 activity may also play a role in the pathobiology of LAM.

To our knowledge, our study was the first to investigate mTOR-related bioenergetic pathways, including glycolysis, oxidative phosphorylation, glutaminolysis, fatty-acid β-oxidation, and acetate utilization in human LAM tissue. High expression of GLS, CPT1A, MCT1, and ACSS2 in our study suggests that glutaminolysis, fatty acid β-oxidation, and acetate utilization may play important roles in LAM cell metabolism. An important role for glutaminolysis has also been shown in TSC2-deficient mouse embryonic fibroblast cells, which are used as an in vitro model for LAM [26, 27]. Some tumors become dependent on acetate or lipids for their growth and survival [28, 29]. Our observation on MCT1, ACSS2, and CPT1A suggests that LAM may be one of these tumors. Moreover, association between CPT1A and ER expression indicates that ERs may affect the lipid metabolism as it has been previously described in breast and endometrial cancer [30, 31].

No statistically significant differences were found between LAM cells and normal BSM cells in GLUT1 and ATPB expression, suggesting that glucose uptake and oxidative phosphorylation are at a relatively low level in LAM cells. The low glucose uptake in our study is consistent with the clinical assessment by lung positron emission tomography scans, that demonstrated low 2-deoxy-2-[18F]fluoro-D-glucose uptake in LAM [14].

Targeting mTOR and various bioenergetic pathways can play an important role in individualized therapy of neoplasms [6-9, 28]. Sirolimus, an mTORC1 inhibitor, has been utilized successfully to attenuate disease progression in patients with LAM [16]; however, there are limitations to the treatment effect as noted in the introduction [17, 18]. Our findings of high mTORC1 and mTORC2 activities in the majority of LAM cases suggest that dual mTORC1/mTORC2 inhibitors may be worthy of clinical investigation, especially in cases with high mTORC2 activity. These cases may be identified by immunohistochemistry for Rictor.

In our study, expression of p-S6 (mTORC1) correlated with that of GLS (glutaminolysis) and expression of Rictor (mTORC2) correlated with that of ACSS2 (acetate utilization) in LAM cells. The former observation is in agreement with recent studies showing that mTORC1 promotes glutamine metabolism [8, 32, 33]. Correlation between mTORC1 and glutaminolysis and mTORC2 and acetate utilization suggests that inhibitors that block mTORC1 and mTORC2 may also have therapeutic effects by blocking glutaminolysis and acetate utilization, which appear to be main sources of LAM cell metabolism. Some LAM-related bioenergetic pathways revealed by our study (eg, acetate utilization) may also represent a promising opportunity for the development of new agents in treatment of LAM.

Interestingly, a relatively low level of hormonal cell surface activity was seen and may partially explain the variable clinical response to changes in either endogenous or exogenous levels. For example, not all patients worsen during pregnancy and there is little improvement with traditional methods of estrogen suppression [34]. Hormonal manipulation may be relegated to an adjunctive role requiring additional study [35].

Our study has some limitations. Although the antibodies used in this study are widely accepted markers of mTOR and metabolic pathways, correlation between immunohistochemical overexpression of proteins and pathway activities requires further investigation. Also, our results are limited to identification of abnormalities without the ability to distinguish cause and effect. Furthermore, hypoxia impacts cellular respiration and, therefore, may play a role in the diversion from aerobic handling of pyruvate to fatty acid metabolism, acetate utilization, and glutaminolysis. Finally, the results indicate potential targets for therapy, but it is unknown whether interventions would result in a clinically meaningful outcome.

In summary, this is the first study to analyze expression of mTORC1, mTORC2 and a set of bioenergetic pathway markers in human lung tissue affected by LAM. If clinically validated, the immunohistochemical panel we have developed may potentially guide clinical interventional trials of new or additional therapeutics for patients suffering from LAM.

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