LAM cells biology and lymphangioleiomyomatosis

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Abstract: Progressive lung tissue destruction in lymphangioleiomyomatosis (LAM) occurs as a result of excessive proliferation of LAM cells caused by a mutation in one of the tuberous sclerosis complex suppressor genes, TSC1 or TSC2. These cells show constitutive activation of the mammalian target of rapamycin (mTOR) pathway and many of the mTOR-related kinases such as Akt, Erk, S6K1 and S6. Phenotype of LAM cells differs considerably depending on their microenvironment. LAM cells show differences in morphology, size and expression of various factors depending on their location in the tumor or body fluids. The presence of LAM cells in blood, urine, bronchoalveolar lavage fluid (BALF), and chyle proves their ability to metastasis. Antigens of smooth muscle cells are expressed in most LAM cells. Some of these cells are immunoreactive with HMB-45 antibody, which is used for the immunohistochemical diagnosis of LAM. Receptors for estrogen and progesterone may also be expressed in these cells, which probably is associated with the fact that LAM occurs almost exclusively in women of childbearing age. LAM cells via increased production of metalloproteinases are involved in the destruction of the extracellular matrix, as well as the remodeling and damage of lung tissue. Sporadic LAM occurs extremely rarely. Therefore a good experimental model of this disease is necessary. To date, several animal and human cell lines, which both genetically and phenotypically resemble LAM cells, have been obtained. These cell lines, derived from LAM nodule or an angiomyolipoma, are usually characterized by a mutation of the TSC2 gene, expression of smooth muscle cell antigens such as a-smooth muscle actin (αSMA) or S6K1 and S6 protein hyperphosphorylation. Presently, there is no commercially available cell line representing a good model of LAM. A better understanding of LAM cell biology is necessary for creating a useful model in vitro for further exploration of both LAM pathomechanisms and more general mechanisms of carcinogenesis. (Folia Histochemica et Cytobiologica 2013, Vol. 51, No. 1, 1–10)

Key words: lymphangioleiomyomatosis, LAM cells, mTOR, TSC1, TSC2, HMB-45, S6K1, αSMA

Abbreviations

4E-BP1 — eukaryotic translation initiation factor 4E-binding protein 1; AKT — protein kinase B; BALF — bronchoalveolar lavage fluid; EGF — epidermal growth factor; EGFR — epidermal growth factor receptor; elf4e — eukaryotic initiation factor-like protein; EMMPRIN — extracellular matrix metalloproteinase inducer; ER — estrogen receptor; ERK — extracellular signal-regulated kinase; HIF-1α — hypoxia-inducible factor 1α; IGF-1 — insulin-like growth factor 1; LAM — lymphangioleiomyomatosis; LOH — loss of heterozygosity; MAPK — mitogen-activated protein kinase; MART-1 — melanoma-associated antigen recognized by T cells; MEFs — mouse embry fibroblasts; MMPs — matrix metalloproteinases; mTOR — mammalian target of rapamycin; PAI-1 — plasminogen activation inhibitor; PDK1 — phosphoinositide-dependent kinase-1; PGE2 — endogenous prostaglandin E2; PgR — progesterone receptor; PI3K — phosphoinositide 3-kinase; PLG — plasminogen; Rheb — Ras homolog enriched in brain;
RhoA — Ras homolog gene family, member A; S6K1 — ribosomal protein S6 kinase beta-1; SRF — serum response factor; STAT3 — signal transducer and activator of transcription 3; TIMPs — tissue inhibitors of matrix metalloproteinases; TORC1 — target of rapamycin complex 1; TORC2 — target of rapamycin complex 2; TRP — tyrosinase-related proteins; TSC — Tuberous Sclerosis Complex; uPA — urokinase-type plasminogen activator; aSMA — smooth muscle actin

Introduction

Sporadic pulmonary lymphangioleiomyomatosis (LAM) is a rare disease, affecting almost exclusively women. It is characterized by intense proliferation of smooth muscle-like cells (LAM cells) in the lungs and around bronchi, blood and lymphatic vessels, which leads to the formation of thin-walled cysts (Figure 1A), the degeneration and remodeling of lung tissue, progressive deterioration of lung function and eventually to death [1–3]. The incidence of sporadic LAM is estimated at 2.6 per 1 million women. LAM may also be associated with an autosomal dominant disease tuberous sclerosis complex (TSC) and occurs in approximately one-third of the women affected by TSC [3]. HAMartoma tumors in various organs such as skin, eyes, kidneys, lungs, and the central nervous system are present in TSC. Sporadic LAM may also give extrapulmonary symptoms, similar to those in TSC, such as renal angiomyolipomas, axial lymphadenopathy, and abdominal lymphangiomatomas [1]. LAM lesions are characterized by an infiltration and accumulation of smooth muscle-like cells. Mutations in either tumor suppressor gene TSC1 or TSC2 cause excessive proliferation of LAM cells and support their ability to metastasis. The phenotype of LAM cells, which include smooth muscle cells appearance with melanoma phenotype is a useful feature in LAM diagnosis [4].

The genetics of LAM and the mTOR pathway

TSC1 and TSC2 belong to the group of tumor suppressor genes, namely the tuberous sclerosis complex. The TSC1 gene is located on chromosome 9q34, and consists of 21 exons encoding 1164 amino acid protein hamartin. The TSC2 gene on chromosome 16p13 contains 41 exons encoding 1807 amino acid protein tuberin [5]. Hamartin and tuberin form a protein complex, which reduces the level of Rheb-GTP through activation of GTP-ase, resulting in an inhibitory effect on mammalian target of rapamycin (mTOR), a highly conserved serine-threonine kinase that plays an important role in the regulation of cell growth and proliferation. Growth factors, phosphoinositide 3-kinase (PI3K) and phosphoinositide-dependent kinase-1 (PDK1) dependent stimulation leads to the phosphorylation and activation of protein kinase B (Akt). Activated Akt, as well as extracellular signal-regulated kinase (ERK), phosphorylates TSC2 resulting in inhibition of its activity as a GTP-ase. Akt is known as an activator of mTOR and is involved in the pathogenesis of many cancer types. Mutation in one of the TSC genes causes the protein complex to be inactivated which promotes mTOR activation leading to the phosphorylation and activation of ribosomal protein S6 kinase beta-1 (S6K1) and S6 ribosomal subunit. This results in the activation of translational...
mechanisms, increased cell growth and proliferation (Figure 2) [6]. Active mTOR kinase phosphorylates eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) which inhibits its interaction with eukaryotic initiation factor-like protein (eIF4E), resulting in the activation of translation and increased cell cycling (Figure 2) [7]. mTOR kinase is a component of two functional complexes: TORC1 and TORC2. TORC1 is the rapamycin-sensitive mTOR complex responsible for the regulation of protein translation initiation and efficiency [8]. Clinical trials with rapamycin as a therapeutic solution for lymphangioleiomyomatosis are in an advanced phase [9]. Rapamycin is a macrolide antibiotic isolated from a strain of *Streptomyces higroscopicus*. It has been already used as an immunosuppressive agent. Rapamycin binds to the cytosolic protein FKBP-12 thereby inactivating the mTOR kinase. Treatment with rapamycin resulted in the reduction of tumor volume and improved lung function in LAM patients [9–11]. However, rapamycin only partially inhibits cell growth, proliferation and disease progression. Yu et al. [8] presented two possible explanations of this fact which may coexist in human LAM. If rapamycin can cause a decrease of cell size and TORC1 inactivation, then a further decrease of tumor size could be obtained, for example, via the inhibition of TSC2-dependent TORC1/rapamycin independent pathway. The second hypothesis assumes that rapamycin can cause a decrease in cell number but some group of cells can still show the TORC1 activation caused by unequal drug delivery or development of rapamycin-resistance. It is hoped that tumor size could be further decreased by using other TORC1 inhibitors, developing a better system of drug delivery or overcoming rapamycin resistance [8].

LAM in women with TSC is associated with germline mutations in *TSC1* or *TSC2* genes. Depending on the type of TSC gene mutation differences in the incidence and severity of pulmonary symptoms have been observed. A significantly higher number of cysts in the lungs was detected in patients with mutations in the *TSC2* gene compared to patients with *TSC1* mutations [12]. It seems that, in particular, changes in the C-terminal segment of tuberin caused by mutations in exons 40–41 of the *TSC2* gene may be associated with LAM symptoms in patients with tuberous sclerosis [5]. Loss of heterozygosity (LOH) of one of the *TSC* genes in somatic cells and clonal expansion of these cells are responsible for sporadic LAM [13,14]. According to the „two hits” Knudson’s theory two independent events lead to the loss of both functional copies of this gene [15]. Sporadic LAM is caused by mutations almost exclusively within the *TSC2* gene. Germline mutations were not observed [16]. There is a significant association between loss of TSC1/TSC2 function and an increase of LAM cells invasiveness and motility. Ras homolog gene family,
member A (RhoA) is a small GTPase protein known to regulate the actin cytoskeleton in the formation of stress fibers. RhoA activity is regulated by TSC1/TSC2 complex formation. TSC2 binds the TSC1 via its binding domain, which overlaps with the RhoA activating domain present in the protein encoded by the TSC1. TSC2 mutation results in the abnormal formation of the TSC1/TSC2 complex and an increase in RhoA activity which increases the invasiveness and migration ability of LAM cells [17]. It was shown that transfection of primary culture of human LAM cells by normal gene TSC2, as well as TSC1 gene silencing resulted in the inhibition of excessive RhoA activity. Loss of TSC2 function by the dysregulation of the TSC1/TSC2 complex formation leads to the TSC1-dependent RhoA activation, an increase of invasiveness, migration, and thus the metastatic nature of LAM cells [17]. The ability to metastasis of TSC2-/− cells can be also explained by the presence of cleaved forms of β-catenin leading to an increased matrix metalloproteinase 7 (MMP7) expression and thus the invasiveness of these cells [18].

**LAM cell phenotype**

LAM cells are morphologically heterogeneous with a phenotype ranging from smaller spindle-shaped smooth muscle-like cells to larger epithelioid-like cells which are abundant with the cytoplasm. Smaller, spindle-shaped cells are located centrally, while epithelioid-like cells are observed mainly at the periphery of the LAM nodule (Figure 1B) [2, 19, 20]. The arrangement of these cells becomes more irregular as the disease progresses [20]. It was suggested that LAM cells proliferation occurs in the central part of the tumor, and then the cells grow intensively, differentiate and migrate to peripheral parts of the tumor [20].

LAM cells show phenotypic features of smooth muscle cells since they express smooth muscle actin (αSMA) (Figure 3A), vimentin and desmin [2, 11, 20,
Increased cell proliferation of ELT3 (epithelioid leiomyoma tumor) cells led to in-shapened cells [26]. Estrogen treatment of TSC2-null LAM cells and in some relatively larger spindle ceceptors was observed mainly in larger epithelioid compared to ER [25, 26]. The expression of both re-frequency and stronger reactivity was seen for PgR clei of LAM cells (Figure 3 C, D). However, greater sion of both receptor types was observed in the nu-
receptors were examined in LAM cells. The expres-
fector (HIF-1α), which is a transcriptional factor for sev-
eral genes during hypoxia, including CXCR4. The expression of some chemokines and their receptors may be partially regulated by hypoxia occurring in patients with moderate or highly advanced LAM. LAM cells also produce large amounts of CCL2. CCL2 and its receptors CCR2 and CCR10 probably play an important role in the pathogenesis of LAM. In vitro studies showed that in a heterogeneous pop-
culation of cells CCL2 was a selective chemoattract-
ant of LAM cells [28]. Interestingly, high expression of this chemokine was also observed in a dominant negative transgenic mouse model of TSC2 [28]. High expression of the chemokines CCL2, CXCL1 and CXCL5 in bronchoalveolar lavage fluid (BALF) of LAM patients as well as in cells derived from LAM nodules was observed. This may suggest their involve-
ment in the pathogenesis of LAM [28]. Clements et al. [29] showed CCR3, CXC4R, CXC6 and CXC3CR1 expression in angiomylipoma and LAM. Ligands for these receptors (CXCL12 CX3CL1, CCL11, CCL24, and CCL28) are responsible for the phosphorylation of Akt and mitogen-activated pro-
tein kinase (MAPK), which promote the mTOR path-
way. Interestingly, CXCL12, the only known ligand for CXC4, was produced by type II pneumocytes surrounding the LAM nodules and vascular endo-
theilum [29]. It may function as a chemoattractant and survival factor for circulating LAM cells. CCR1, CCR7, and CXC7 mRNA were detected in angio-
mylipoma primary cell culture [29]. Expression of the specific chemokine profile in LAM was shown to affect LAM cells migration, metastasis, tumor pro-
gression and interaction between LAM and stroma cells [28–30].

Due to the fact that LAM almost exclusively a-
flicts women, estrogen (ER) and progesterone (PgR) receptors were examined in LAM cells. The expres-
sion of both receptor types was observed in the nu-
ci of LAM cells (Figure 3 C, D). However, greater frequency and stronger reactivity was seen for PgR compared to ER [25, 26]. The expression of both rece-
ptors was observed mainly in larger epithelioid LAM cells and in some relatively larger spindle shaped cells [26]. Estrogen treatment of TSC2-null ELT3 (epithelioid leiomyoma tumor) cells led to in-
creased cell proliferation in vitro and significantly enhanced the metastasizing ability of these cells in vivo [27]. Although the first therapeutic trials were based on suppression of estrogen activity, the efficacy of these therapies has not yet been proven. Migration and behavior of LAM cells depends on the activities of specific chemokines. These cells, like other cancer cells, have a characteristic expression profile of chemokines and their receptors. High expression of CXCR4 receptor was detected in LAM cells. Loss of TSC2 function, through activation of mTOR, can lead to the increased expression of hypoxia-inducible fac-
tor (HIF-1α), which is a transcriptional factor for sev-

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**Note:** The text appears to be a continuation of a scientific discussion, possibly about LAM cell biology and lymphangioleiomyomatosis.
tial transcriptional factor which is highly expressed in developing smooth muscle cells, however, its expression decreases in mature cells [32]. LAM cells are morphologically similar to immature myoblasts and expression of SRF was observed in both nuclei and cytoplasm. High SRF level increased expression of MMP-2 and MMP-14 and reduced expression of one of their inhibitors, the metalloproteinase inhibitor 3 (TIMP-3) [32]. The lower level of TIMP-3 may be of particular significance in the progression of LAM since TIMP-3-null mice showed a destructive lung phenotype, which led to progressive emphysema and a shorter life span [32]. A study with the overexpression of MMPs in human lung fibroblasts demonstrated that high levels of SRF may also regulate the plasminogen system in vitro [33]. SRF, by increasing levels of urokinase-type Plasminogen Activator (uPA), activates plasminogen (PLG) to plasmin, which in turn activates MMPs. A high level of SRF reduces the expression of plasminogen activation inhibitor (PAI-1). These results [33] were confirmed by laser micro-dissection, RT-PCR, and immunohistochemistry on sections of lung tissue from LAM patients. Strong positive immunohistochemical reactions with uPA and PLG were observed in LAM nodules compared to the surrounding healthy lung tissue. PAI-1 expression was observed in normal lung tissue, whereas it was not present in LAM lesions [33]. Another protein which was shown to regulate the activity of MMPs, extracellular matrix metalloproteinase inducer (EMMPRIN/CD147), was detected in LAM cells. Double immunofluorescence revealed co-localization of EMMPRIN/CD147 and αSMA, MMP-2 or MMP-9. Levels of EMMPRIN/CD147 were significantly elevated in LAM lesions and BALF of LAM patients [34]. High expression of MMPs in TSC2± angiomyolipoma cells seems to be independent of mTOR kinase and their levels do not change following rapamycin treatment, therefore the treatment of LAM by this mTOR inhibitor may have limited clinical effectiveness [35]. It has been hypothesized that doxycycline, as an inhibitor of MMPs, could inhibit degradation of lung tissue in LAM patients. Clinical trials have shown that administration of doxycycline to LAM patients resulted in lower levels of MMP-2, MMP-9 in serum and urine, decreased symptoms and improved life quality [36, 37]. However, in vitro studies showed that there was no significant reduction in the level and activity of MMPs in the ELT3± cells, derived from an animal model of LAM, the Eker rat, after doxycycline cells treatment [38]. Thus, the efficacy of doxycycline to inhibit MMPs activity in LAM patients needs to be further investigated.

Many of the above described features of LAM cells reveal their ability to metastasize. So far, the most useful marker of these cells, commonly used in the differential diagnosis of LAM is an immunohistochemical reaction with the antibody HMB-45. Exploring the biology of LAM cells, in particular characterization of disseminated neoplastic LAM cells in the body fluids, could lead to a development of new diagnostic tools which would be less invasive than a biopsy. In order to find specific markers for LAM cells circulating in blood, urine or BALF, Pacheco-Rodriguez et al. [39] carried out a series of molecular studies. Analysis of blood samples from LAM patients showed that cells with TSC2 LOH were immunoreactive with anti-CD235a antibody against glycoporphin-A, a sialoglycoprotein present in erythrocyte’s cell membrane. The expression of this protein was also demonstrated in 31% of lung LAM cases. This protein was also reported to be present on the surface of breast cancer and melanoma cells [39]. It was also found that cells from the nodule express CD44v6, the product of CD44 alternative splicing [40]. CD44 is a class I transmembrane glycoprotein. The formation of CD44 isoforms is regulated by a signaling pathway involving the Ras-mitogen activated protein kinase pathway [40]. Isoforms generated by alternative splicing of 10 additional exons from v1 to v10 resulted in an insertion of additional segments in the extracellular domain of the protein. The extracellular domain of CD44 binds metalloproteinases such as MMP-7 and MMP-9, which cleave CD44 and CD44v6 [40]. CD44v6 is associated with carcinogenicity and is involved in homing during metastasis, and its expression was demonstrated on squamous cell carcinoma and adenocarcinoma cells [40]. The CD44v6 domain can also bind Fas ligand, which inhibits apoptosis. LAM lesions showed moderate reaction with CD44, however, the usefulness of this antigen for LAM cells isolation from body fluids is limited because it shows a strong immunoreactivity with other cell types such as vascular smooth muscles cells, bronchial epithelial cells and hyperplastic type II pneumocytes [40]. A large percentage of cells in the LAM nodule showed expression of CD44v6 in contrast to other cells in the lung tissue. CD44+/CD44v6+ cells from LAM nodule, exhibit LOH of the TSC2 gene [40]. The presence of CD44v6 was found on LAM cells isolated from BALF, urine, and chyle [41]. Microarray analysis performed on material derived from the skin tumor associated with TSC showed high expression of CD9, a protein of the tetraspanin family which plays an important role in cell morphology, motility, invasiveness, adhesion as well as cellular interactions [41]. Its expression may correlate with metastases of tumor cells. A higher level of CD9 was correlated with TSC2 LOH [41]. Both CD44v6 and CD9 are useful markers to distinguish and isolate LAM cells from...
Table 1. Mechanisms of metastasis in lymphangioleiomyomatosis

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<th>Mutations and markers in LAM cells</th>
<th>Mechanisms</th>
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<tr>
<td>Loss of heterozygosity of TSC2</td>
<td>Constitutive activation of mTOR pathway, enhanced cell growth and proliferation, cells survival [13, 14]</td>
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<tr>
<td>Estrogen receptor, ER</td>
<td>Estrogens promote proliferation, survival and lung colonization of ELT3 cells, activation of MMPs [27]</td>
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<tr>
<td>Chemokines and its receptors e.g. CCL2,CCR2, CXCR4</td>
<td>Enhanced migration, homing, and interactions between LAM and stroma cells [28–30]</td>
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<tr>
<td>Metalloproteinases, MMPs e.g. MMP-2, MMP-9</td>
<td>Degradation of extracellular matrix which facilitates LAM cells invasion [27]</td>
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<td>CD44, CD44v6</td>
<td>Homing, inhibition of apoptosis by binding Fas ligand [40]</td>
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<td>CD9</td>
<td>Motility, invasiveness, adhesion, cells interactions [41]</td>
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body fluids. CD44v6+/CD9+ cells showed TSC2 LOH in 80% in BALF, 69% in urine and 50% in chyle [41]. Changes in metastatic cell phenotype can occur depending on the microenvironment. The LAM cells in different locations may exhibit different antigen expression patterns such as CD44/CD44v6 expression in LAM cells from the LAM nodule, CD235 in LAM cells in blood and CD44v6/CD9 expression in LAM cells in urine, BALF, and chyle [41].

Metastatic features of LAM cells are presented in Table 1.

Cell lines as a model of LAM

Lymphangioleiomyomatosis is a rare interstitial lung disease. An applicable experimental model is still required for carrying out further studies. The most popular animal model of LAM is the Eker rat with TSC2 germline mutation occurring in one of its alleles. In this model rats develop renal adenomas, uterine leiomyomas and pituitary adenomas. In some of them loss of heterozygosity for the TSC2 gene may be observed. Heterozygous TSC2+/− mice develop changes similar to those observed in the Eker rat, and some of them were characterized by TSC2 LOH. In TSC1+/− mice a similar phenotype was seen, however, kidney tumors developed with a lower frequency [42]. Germline inactivation of both alleles of TSC1 or TSC2 in mice is lethal [43].

The most commonly used cell lines derived from an animal model of LAM are TSC2-null lines derived from tumors present in the Eker rat, ELT 3, 4, 6, 9, 10. These lines, isolated from uterine leiomyomas, exhibit expression of smooth muscle antigens and constitutive activation of mTOR kinase. All of these cell lines express receptors for estrogen and progesterone, however, only ELT6 was shown to respond to these hormones in culture [44]. Although TSC1 and TSC2 knockouts are lethal, a cell line derived from embryonic TSC2-null Eker rat, named EEF-8, was obtained, as well as TSC2+/− fibroblasts derived from this model [45]. Mouse embryo fibroblasts (MEFs) isolated from both the TSC1 and TSC2 knockout mice exhibited constitutive activation of mTOR and S6K1 phosphorylation [46, 47].

In 2001, Arbiser et al. [48] generated a cell line from human sporadic angiomyolipoma of a 63-year-old patient which was not associated with TSC. Cells were transfected with SV40 large T antigen and human telomerase to obtain a stable and immortalized cell line. This cell line expressed tuberin and hamartin, however, it developed an increased activation of MAPK. There was a positive immunohistochemical reaction with cytokeratins which suggests that this culture can represent epithelioid-like cells. This cell line is commercially available [48].

Human cell lines were successfully isolated from tissue obtained from patients with angiomyolipoma in Tuberous Sclerosis Complex. Lesma et al. [49] characterized two cell lines derived from angiomyolipoma of a 42-year-old patient with TSC. Cells were characterized by immunocytochemistry showing high expression of αSMA and HMB-45 antibody immunoreactivity. However, staining with S100, vimentin, CD68 and keratins 8/18 was negative. The second cell population, epithelial-like cells, strongly reacted with the antibody against cytokeratins 8/18 and HMB-45 and did not express α-SMA, S-100, vimentin or CD68. Epithelial-like cells (R+) strongly reacted with RhoA antibody but did not show LOH for the TSC2 gene [49]. Smooth muscle-like cells (A+) revealed TSC2 LOH and a lack of tuberin expression. A+ cells had receptors for epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF-1) on their surface and EGF medium supplementation was essential for the survival and proliferation of these cells in culture [49].
These cell lines were used to study the effects of monoclonal antibody anti-EGFR, rapamycin and other mTOR pathway blockers on cell survival. It was found that anti-EGFR antibody effectively inhibited the proliferation of these cells suggesting its use as an effective therapeutic option [50]. A study of the role of survivin as an inhibitor of apoptosis in these cell lines showed that it may be an important anti-apoptotic and survival factor with the potential of becoming the target of future therapies [51]. Clements et al. [52] demonstrated two cell line populations derived from angiomyolipoma: spindle-shaped cells and epithelioid-like cells. The cultured cells showed constitutive activation of S6K1 protein and strong expression of αSMA, mRNA of gp100, and MART-1 (spindle-shaped cells) and estrogen receptors was detected in these cells by RT-PCR. Cells were cultured in the presence of epidermal growth factor [52] and used to study the role of chemokines in LAM and angiomyolipoma [29] as well as the effects of doxycycline on the proliferation, production of MMPs and adhesion of LAM-related cells [38]. Yu et al. [53] described a line derived from human angiomyolipoma. The cells were spindle-shaped, showed LOH for TSC2, the expression of estrogen receptors and hyperphosphorylation of S6. Both estradiol and, unexpectedly, tamoxifen, stimulated their growth demonstrating that tamoxifen may act as an estrogen agonist in human cultured angiomyolipoma cells [53], in opposition to being an estrogen antagonist in Eker rat-derived ELT3 cells [54]. All human angiomyolipoma cells were used between the second and fourth passage in this experiment [53]. The same, but immortalized human angiomyolipoma cell line, was used to study the level of MMP-2 expression and the mechanism leading to its overexpression [35].

Black et al. [55] studied LAM cells isolated from a fragment of lung tissue using laser micro-dissection. They were immunohistochemically characterized as HMB-45 positive. All cells in this experiment were used between the fourth and eighth passage. The authors found reduced expression of endogenous prostaglandin E2 (PGE2) associated with the decreased expression of the cyclooxygenase 2 (COX2). Production of the VEGF protein family by these cells was documented [55]. Goncharova et al. [56] obtained LAM cell lines from the LAM nodule of lung tissue derived from patients after lung transplantation. Lines were characterized as αSMA positive, and HMB-45 negative. These cells showed constitutive hyperphosphorylation of p70S6, S6 protein and high proliferative activity, even without growth factor supplementation. Cells were used between the 3rd and 12th passage. This study showed that TSC2 dysfunction and constitutive activation of mTOR/S6K1 attenuates growth-inhibitory effect of IFN-β and suggests that combination of rapamycin and IFN-β cells treatment could abrogate LAM cells proliferation [56]. The same authors have next demonstrated that activation of signal transducer and activator of transcription 3 (STAT3) was essential for the proliferation and survival of this cell line [57].

Conclusions

LAM cells are a heterogeneous population of cells. Their proliferation leads to the formation of LAM lesions, cysts and lung tissue destruction. Differences in size, morphology and antigen pattern expression, make the identification and isolation of LAM cells very difficult. The phenotype of these cells depends on their microenvironment, exposure to cytokines, growth factors and interactions with other cells. A better understanding of the nature of LAM cells is necessary in order to find an applicable in vitro model, as well as new therapeutic modalities. Analyses of genetic abnormalities or characteristic markers in blood or other body fluids, such as BALF, chyle or urine could provide less invasive methods for early diagnosis of LAM.

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