A Current Viewpoint of Lymphangioleiomyomatosis Supporting Immunotherapeutic Treatment Options

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Lymphangioleiomyomatosis (LAM) leads to hyperproliferation of abnormal smooth muscle cells in the lungs, associated with diffuse pulmonary parenchymal cyst formation and progressive dyspnea on exertion. The disease targets women of child-bearing age. Complications include pneumothoraces and chylous pleural effusions. Ten-year survival is estimated at 70%, and lung transplantation remains the only validated treatment. It has been observed that LAM cells express markers associated with melanocytic differentiation, including gp100 and MART-1. Other melanocytic markers have also been observed. The same proteins are targeted by T cells infiltrating melanoma tumors as well as by T cells infiltrating autoimmune vitiligo skin, and these antigens are regarded as relatively immunogenic. Consequently, vaccines have been developed for melanoma targeting these and other immunogenic melanocyte differentiation proteins. Preliminary data showing susceptibility of LAM cells to melanoma-derived T cells suggest that vaccines targeting melanosomal antigens can be successful in treating LAM.

Keywords: lymphangioleiomyomatosis; TSC1; TSC2; melanoma associated antigens; immunotherapy

LYMPHANGIOLEIOMYOMATOSIS DISEASE DEMOGRAPHICS

Lymphangioleiomyomatosis (LAM) leads to hyperproliferation of abnormal cells in the lungs, associated with diffuse pulmonary parenchymal cyst formation and progressive dyspnea on exertion (1). Patients often develop pneumothoraces and chylous pleural effusions, correlating in part to the severity of disease (2). Ultimately, supplemental oxygen is required (3). Lung transplantation remains the sole validated treatment, with one third of diagnosed patients transplanted or waitlisted for lung transplantation at any time (4). LAM adheres to the common criteria of cancer, including tumor formation, uncontrolled and growth-factor–independent growth of affected cells in culture, and lymphatic metastasis, yet the tumors qualify as relatively benign (3). LAM has been associated with tuberous sclerosis complex (referred to as TSC-LAM), with more than a third of patients with TSC having typical cysts noted on computed tomography (5). The mean age of patients at diagnosis is 36 years (6). The disease almost exclusively strikes women in the prime of their life, although sporadic cases of LAM in men have been described, and a very small percentage of male patients with TSC do develop LAM (5). Progression can be accelerated by pregnancy and hormonal contraception, suggesting hormonal involvement in disease pathophysiology, an idea further supported by estrogen and progesterone receptor expression in affected lungs (7). By its recognized prevalence of almost one per million, it officially qualifies as a rare disease, yet a significant cohort of female patients affected by TSC ultimately develop LAM, suggesting that LAM remains vastly underdiagnosed (8). This concept is supported by a recent surge in patients diagnosed with LAM in Korea, reported after enhanced screening methods (9). LAM is inheritable, in particular the TSC-associated form. LAM also carries a strong association with renal angiomyolipoma, and abdominal pain is among the initial symptoms reported by patients.

LAM ETIOLOGY

Breakthrough advances were made with the discovery of mutations in *TSC1* or *TSC2* as underlying causes for LAM (10). Gene products hamartin and tuberin function as heterodimers; hence, mutations in either gene define the same disease(s) (11). The association between both gene products has been mapped to amino acids 302–430 in hamartin and amino acids 1–418 in tuberin (12). This association is required to prevent ubiquitination and premature degradation of the *TSC2* gene product, which eliminates the GAP activity of tuberin (13). Several different point mutations have been described, primarily in the C-terminal GAP site or in the regions affecting interactions among both proteins (13, 14). Mutant tuberin is unable to control the small GTPase Rheb, and subsequent mTOR activation accompanied by hyperphosphorylation of S6 ribosomal protein leads to increased cell growth (15–17). At the same time, Rheb negatively affects differentiation by inhibiting B-Raf (18). In TSC-LAM, a disease with autosomal dominant inheritance, a mutant copy of *TSC1* or *TSC2* is inherited through the germline (19). Tumors result from inactivation of the second allele of either gene by loss of heterozygosity or promoter methylation (20). Patients can thereby lose functional expression of hamartin or tuberin, respectively, in tissue cells, affecting a multitude of organs that are dependent on the wide variety of binding partners for either gene product (21). Tuberous sclerosis carries a prevalence of approximately 1:10,000 (19). Not all patients with TSC develop LAM, implying that additional mutations or environmental cues are required for disease expression. Also, the fact that mutations in genes with products playing such a central role in cell proliferation and cell growth have less profound effects than mutations more upstream can likely be assigned to simultaneous attenuation of Akt (22). Indeed, mTOR inhibition in mice with mutations in PTEN leads to much more aggressive tumors (23). The same pathway also

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leads to STAT3 overexpression, implicated in tumor hyperproliferation in LAM (24). In tuberous sclerosis, patients can present with neurological disorders, including epilepsy, autism, and mental retardation, and with tumors in multiple organs, including renal angiomyolipomas, ash-leaf shaped depigmentation patterns of the skin, and pulmonary LAM (25). Lung lesions leading to TSC-associated or sporadic LAM are often described as hyperproliferative smooth muscle cells (25). Whether causative cells originate in the lung or metastasize through the lymphatics is a topic of debate (26–28). Support for the metastatic ability of LAM cells is provided by a demonstrated role for tuberin in cell motility (29). Metastasis involves estrogen receptor activation followed by overexpression of matrix metalloproteinases in LAM (30–34). Further answers may come from sporadic LAM, where multiorgan involvement in patients is suggestive of metastatic events. Enhanced expression of metastasis-associated CD44 splice variant 6 in LAM lesions is likewise supportive of a common origin of disease-associated cells in LAM and renal lesions (35).

CURRENTLY AVAILABLE TREATMENTS

Preventing further outgrowth of hyperproliferative lesions is a main objective of LAM treatment, and the discovery of *TSC1* and *TSC2* mutations in LAM has opened avenues to treatment because mTOR activation can be subjected to rapamycin analogs (36). Symptoms did not progress during rapamycin treatment, yet progression resumed after treatment was halted (37). Although cell proliferation is inhibited and cell size is reduced, existing tumor cells are not killed by the treatment, so additional treatment modalities are required for long-term benefit. Because rapamycin supports cellular autophagy, it has been proposed that adding inhibitors of this survival-promoting process will improve the effects of rapamycin (38). Recent findings demonstrating that *TSC2* mutations affect not only mTORC1 but also mTORC2 activity indicate that outcomes may also improve by combinatorial treatment with simvastatin (39, 40). Treatment with rapamycin alone can be effective in a prophylactic setting in patients with LAM after undergoing lung transplantation (41). The high risk of the operation and the limited supply of donor organs, however, limit the applicability of transplantation as an option for LAM. Moreover, rapamycin has potent immunosuppressive consequences that can be further potentiated by additional treatment to prevent tissue rejection in transplant recipients (42).

LAM DIAGNOSIS

Among the initial symptoms recognized by patients is abdominal pain and shortness of breath (43). However, the mean time elapsing between such initial symptoms and official diagnosis is 8 years (44). Survival is estimated at 70% after 10 ears (44). A definitive diagnosis generally requires confirmation by a lung biopsy further analyzed for immunostaining by antibody HMB45 (human melanoma black 45), reactive with gp100, a melanosomal glycoprotein otherwise exclusively expressed in cells of the melanocyte lineage (45). In the future, antibodies to β-catenin may be used to further support the diagnosis of LAM (46). Serum VEGF-D levels can serve as a reliable marker for LAM, and, currently, the combination of a VEGF-D level $>$ 800 pg/ml along with the finding of lung cysts on CT establishes the diagnosis of LAM without the need for surgical lung biopsy (27, 47, 48).

IDENTITY OF LAM CELLS

In human LAM, features of hyperproliferative cells suggest a smooth muscle origin of LAM lesions (49). However, expression of smooth muscle actin is not unique to smooth muscle cells. Lesional LAM cells also frequently express receptors for the female hormones estrogen and progesterone (50). Some have suggested an endothelial cell origin, supported by the observation that lesional cells in *Tsc1* heterozygote mice may be of endothelial origin (51). The definitive marker for LAM cells is recognition by antibody HMB45, supporting gp100 expression by at least a subset of LAM cells in all patients and suggesting a melanocytic origin of transformed cells in LAM (52). Given the current speculation about the origin of the LAM cells, it is hypothesized that LAM cells originate from a cancer stem cell, which is able to acquire characteristics of multiple lineages upon differentiation. Prominent expression of gp100 inversely correlates with cell proliferation. This suggests that a patient with more aggressive disease would exhibit fewer LAM cells expressing gp100 because rapidly proliferating cells do not react with HMB-45 (53). Our own studies suggest that gp100 expression is antiapoptotic because cells transduced to express gp100 also overexpress Bcl-2 (54). Evidence for a more extensive melanocytic differentiation program within LAM cells is provided by expression of MART-1 and CD63 (tetraspanin) (53). LAM tumor cells can also express melanoma-associated antigens and tyrosinase-related proteins

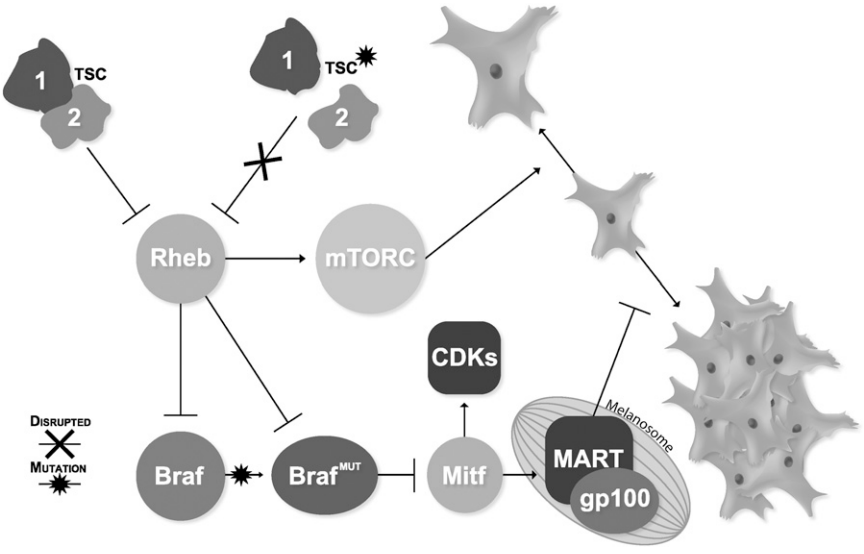

Figure 1. A central role for Rheb in dysregulating cell size and differentiation profile in lymphangioleiomyomatosis LAM? When *TSC1* or *TSC2* are mutated, the tuberous sclerosis complex (TSC) is incapable of inhibiting Rheb, which leads to activation of mTORC, supporting an increase in cell size. Uninhibited Rheb simultaneously leads to inhibition of wild-type and mutant Braf. At least in the case of mutant Braf, this releases repression of Mitf, increasing CDK expression and cell cycling as well as expression of melanoma-associated antigens in melanosome-like organelles. Unknown factors remain, yet these conditions likely contribute to aberrant expression of melanoma antigens in LAM. This profile can render LAM tumor cells susceptible to vaccines designed to target malignant melanoma.

(TRP)-1 and TRP-2, and detection of TRP-1 may be a more reliable indicator of LAM than gp100 (55). By contrast, melanin formation is absent, and antibodies to the associated enzyme tyrosinase were not reactive with LAM tissue or angiomyolipoma of the lung in a patient with TSC (55, 56). Ultrastructural studies confirm the presence of premelanosomes in the absence of melanin deposition (55).

A LINK WITH MELANOMA

Melanoma cells are not known to carry mutations in *TSC1* or *TSC2*, although mTOR activation is a common feature and mTOR inhibition by rapamycin combined with PI3 kinase inhibitors has met with therapeutic success in animal models of melanoma (57, 58). In melanoma, malignancy is associated with early mutations in BRAF, the gene product of which is suppressed by Rheb (59). Although mutations affecting the tuberin/hamartin complex lead to increased Rheb activity and therefore to elevated mTORC activity and cell growth, increased Rheb also inhibits BRAF-induced differentiation (Figure 1). However, only oncogenic BRAF can (negatively) regulate Mitf expression (60). Mitf is required for melanocytic differentiation and is responsible for the expression of gp100 and MART-1 (61). Thus, it may be predicted that BRAF mutations, frequently observed in melanoma, are uncommon in LAM (62). In melanoma, mutant BRAF-induced suppression of Mitf is countered by a requirement for Mitf to induce expression of CDKs, including CDK2, which shares a promoter with gp100 (54, 60). The observation that TORC1 signaling affects melanosome formation may shed light on the underlying mechanism driving melanosomal antigen expression in LAM (63). A hallmark of malignantly transformed melanocytes in melanoma is their continued expression of differentiation antigens gp100 and MART-1, which are among the most immunogenic tumor-associated antigens known. In fact, MART-1 stands for Melanoma Antigen Recognized by T cells, and the majority of T cells infiltrating melanoma tumors are reactive with MART-1and gp100 (64). Other melanocyte differentiation antigens expressed in LAM are also immunogenic, including TRP-1 and TRP-2 (64). Immunogenic proteins expressed by melanoma cells also include gene products associated with malignant transformation, such as the MAGE family of proteins, and GD3 (65). What makes melanoma such a uniquely immunogenic tumor is the melanosome. This organelle carries physiologic properties otherwise associated with lysosomes, including processing of antigens to be presented in the context of MHC class II (66). Because primary melanocytes only express MHC class II molecules under pathologic conditions, the immune system may be tolerant to melanosomal peptides due to ignorance (67). In the autoimmune disease and in malignant melanoma, however, melanocytes or their malignant counterpart, the melanoma cell, do express class II molecules; this may explain why tolerance to melanosomal self-proteins is broken.

IMMUNOTHERAPY TARGETING MELANOMA-ASSOCIATED ANTIGENS

Rare observations of spontaneous remissions among patients with melanoma have sparked interest in the underlying mechanism. Cells mediating cytotoxicity toward tumor cells were isolated and grown in bulk and then adoptively transferred back to patients (68). Original strategies have since been refined, boosting immune responses with vaccines containing melanosomal target antigens in the form of DNA, RNA, protein, or peptides in natural or modified format as presented in the context of HLA, involving dendritic cells (69). Further immunotherapeutic developments include T-cell receptor transgenic T cells, antibodies and anti-idiotype antibodies, and even chimeric antigen receptors combining high-affinity antibody paratopes with T-cell signaling advantages (70). Cytokines, costimulatory molecules, heat shock proteins, and other adjuvants as well as combinations with cytostatic drugs are included in current vaccine strategies to target melanoma (71, 72). Tregs have since been recognized as major impediments to effective tumor targeting, and antibodies to CD25 are used to deplete Tregs (73). Whereas the window of opportunity for successful treatment of advanced stage melanoma remains limited, similar strategies may be applied with more success in less aggressive LAM tumors expressing the same target molecules. This concept is strongly supported by the observation that LAM cells cultured from affected lung tissue were susceptible to melanoma-derived cytotoxic T cells to an extent well beyond that predicted based on detectable gp100 expression (55). The latter observation supports the concept that, in cells lacking mature melanosomes, molecules otherwise destined for the melanosome are driven to the endosomal compartment to favor antigen processing and presentation (74). Despite elevated expression of the T-cell costimulatory molecule B7H3, the expression of immunogenic melanoma-associated antigens in LAM is not accompanied by indications of enhanced T-cell infiltration of tumor tissue (75). Thus, intrinsic immunosurveillance of LAM tumors is inadequate to keep slow tumor growth in check. At the same time, infiltration by increased numbers of tumor-promoting macrophages has been observed in TSC lesions and LAM lung (55, 76). Taken together, these findings suggest that existing and enhanced vaccines developed to treat malignant melanoma may be suitable for treating LAM.

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