β-Catenin Is a Useful Adjunct Immunohistochemical Marker for the Diagnosis of Pulmonary Lymphangioleiomyomatosis

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Abstract

Lymphangioleiomyomatosis (LAM) is a rare multisystem disease that affects women of reproductive age. LAM can occur sporadically or in association with the genetic disorder tuberous sclerosis complex (TSC). Although the clinical manifestations and progression of LAM are variable, women with LAM typically have progressive dyspnea and recurrent pneumothoraces due to cystic destruction of the lung parenchyma. In addition to the pulmonary manifestations of LAM, lymphadenopathy, lymphangioleiomyomas, and renal angiomyolipomas are also observed, with renal angiomyolipomas occurring in an estimated 40% to 50% of patients with sporadic LAM.

Histologically, the lungs of patients with LAM contain the characteristic thin-walled cysts and LAM cells. LAM cells can be found diffusely distributed throughout the lung parenchyma and/or in multifocal LAM-cell nodules. LAM cells express desmin and α-smooth muscle actin and are, therefore, considered to be atypical (ie, immature) smooth muscle cells. In the majority of cases, a subset of LAM cells also expresses the melanocytic marker HMB45. Indeed, it is the combination of immunopositivity for smooth muscle actin and HMB45 that ultimately determines a pathologic diagnosis of LAM in the cases for which a diagnosis cannot be definitively made by high-resolution computed tomography scanning combined with genetics and/or additional lung or extrapulmonary characteristics.

Recently, the European Respiratory Task Force published guidelines for the diagnosis and management of LAM. According to these recommendations, the pathologic criteria for a LAM diagnosis in lung biopsies should include a variable predominance of cysts and multifocal, nodular, proliferating smooth muscle and perivascular epithelioid cells.
Furthermore, when morphologic characteristics do not allow for a definitive diagnosis, immunohistochemical analysis for α-smooth muscle actin and HMB45, and possibly estrogen receptor α (ER-α) and progesterone receptor (PR), should be performed. Evidence demonstrates the potential role of aberrant β-catenin signaling in TSC, and β-catenin was shown to be highly expressed in 7 sporadic LAM cases. To further understand the pathogenesis of LAM and to examine the diagnostic usefulness of β-catenin, we performed immunohistochemical analysis for established LAM cell markers, HMB45, ER-α, and PR, and β-catenin on 28 pulmonary LAM cases and 10 cases of renal angiomyolipoma resected from patients with sporadic LAM. Our findings confirm the results of initial preliminary studies and show that all 28 LAM cases evaluated express high levels of β-catenin, suggesting that it is as good as if not a better marker than HMB45 for LAM.

Materials and Methods

Case Selection

Ethical approval for the study was obtained from the Dana Farber Institutional Review Board (protocol No. 07-015; Boston, MA). LAM and angiomyolipoma cases numbered in the 9000s were obtained from the National Disease Research Interchange Rare Disease Program (http://www.ndiresource.org/NDRI_Initiatives/Rare_Disease/30/). A total of 38 cases including 28 cases of pulmonary LAM and 10 renal angio- myolipomas were studied. All cases were formalin-fixed and paraffin-embedded following lung and renal biopsy for diagnosis or were obtained at autopsy. Control samples included 6 cases of chronic bronchitis and pulmonary emphysema, 5 cases of Langerhans cell histiocytosis, and normal lung parenchyma adjacent to the lesional tissue. Control samples were retrieved from the Departments of Pathology, The Brigham and Women’s Hospital, Boston, MA, and from the Pathology Unit, Addari Institute of Oncology, Bologna, Italy. H&E-stained slides of all cases were reviewed by 2 histopathologists (R.J.F. and M.F.) and original diagnoses confirmed. Histologic studies demonstrated the characteristic appearance of cystic air spaces and a patchy, disordered nodular proliferation of bland spindle and epithelioid smooth muscle cells around airways, lymphatics, and blood vessels Image 1. LAM cells were confirmed by immunohistochemical staining for hormone receptors ER-α and PR and the melanocytic marker HMB45 Image 2.

Immunohistochemical Analysis

For the study, 4-μm serial sections were cut and mounted on glass slides. For antigen unmasking, heat-mediated antigen retrieval was performed on deparaffinized sections using citrate buffer (10 mmol/L sodium citrate buffer [pH 6.0]) before incubation with primary antibodies. HMB45, ER-α, PR, and β-catenin (DakoCytomation, Copenhagen, Denmark) protein levels were examined using mouse monoclonal antibodies at dilutions of 1:50, 1:25, 1:25, and 1:200 respectively. The immunoreactions were revealed by using the BioGenex Super Sensitive Link-Label IHC Detection System (BioGenex, San Ramon, CA). For negative control samples, we replaced primary antiserum with nonimmune bovine serum. Chromogen detection was performed with diaminobenzidine (DAKO, Carpinteria, CA) solution (0.5 ml of stock diaminobenzidine in 4.5 mL of tris(hydroxymethyl) aminomethane buffer with 20 μL of hydrogen peroxide). Slides were counterstained with hematoxylin.

Two pathologists (R.J.F. and M.F.) blinded to the original diagnosis scored the sections independently. Nuclear immunoreactivity for ER-α and PR, cytoplasmic staining for HMB45, and membranous-cytoplasmic staining for β-catenin were semiquantitatively evaluated as to their variable intensity and scored as follows: 0, negative; 1, weak; 2, moderate; or 3, strong. At least 10 high-power fields corresponding to approximately 2,000 cells were evaluated for each case. In case of different intensity within the same case, the average mean intensity among the high-power fields was calculated.

Statistical Analysis

All statistical analysis of immunohistochemical studies was performed with Analyse-It Software (Analyse-It Software, Leeds, England). Multiple comparisons were performed with the Kruskal-Wallis test. A χ test was used as a measure of the agreement between the 2 study histopathologists for each immunohistochemical stain. All tests were 2-tailed, and the significance level was set at a P value of .05 or less.

Results

Pulmonary LAM Cases

All 28 cases (100%) of LAM were positive for β-catenin, and the mean immunoreactivity was 2.42 (24/28 cases [86%] showed immunoreactivity ≥2) Table 1. Spindle- and epithelioid-shaped LAM cells showed predominantly diffuse homogeneous cytoplasmic and membranous staining for β-catenin (Image 2) and coexpression with HMB45, ER-α, and PR. All 28 cases (100%) of LAM were positive for HMB45, and the average immunoreactivity was 1.57 (8/28 cases [29%] showed immunoreactivity ≥2; Table 1). Of the 28 cases of LAM, 20 (71%) were positive for ER-α, and the average immunoreactivity was 0.91 (3/28 cases [11%] showed immunoreactivity ≥2).

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In 27 (96%) of 28 cases of LAM, there was positivity for PR, and the average immunoreactivity was 1.94 (18/28 cases [64%] showed immunoreactivity ≥2; Table 1). The mean immunoreactivity for β-catenin was greater than that observed for HMB45, ER-α, and PR, and the difference in immunoreactivity between all 4 immunohistochemical markers was statistically significant (P < .0001; Kruskal-Wallis test).

Control Cases and Specificity

We assessed the specificity of β-catenin for LAM in 5 cases of Langerhans cell histiocytosis, 6 cases of chronic bronchitis and emphysema, and normal lung tissue adjacent to the lesional tissue. Langerhans cells in all 5 cases of histiocytosis X were negative for β-catenin. There was expected background membranous staining for β-catenin in bronchial epithelium, type 2 pneumocytes, and occasional endothelial cells. It is important to note that perivascular and peribronchial smooth muscle cells and normal lung parenchyma were negative for β-catenin.

Agreement Between Histopathologists

The measure of agreement between the 2 study pathologists was assessed following independent and blinded review.
of the study set. The level of agreement between observers for β-catenin was comparable to that for HMB45 (observed agreement, 1.00 and 0.964, respectively) Table 2.

Renal Angiomyolipoma Cases

In 8 (80%) of 10 cases of angiomyolipoma, there was positivity for β-catenin, and the mean immunoreactivity was 1.58 (6/10 cases [60%] showed immunoreactivity ≥2) Table 3. Spindle and epithelioid smooth muscle and adipocytic cells showed cytoplasmic and membranous staining. All 10 cases (100%) of angiomyolipoma were positive for HMB45, and the average immunoreactivity was 2.63 (9/10 cases [90%] showed immunoreactivity ≥2; Table 3). Of the 10 cases of angiomyolipoma, 1 (10%) was positive for ER-α, and the average immunoreactivity was 0.15 (0/10 cases [0%] showed immunoreactivity ≥2; Table 3). In 5 (50%) of the 10 cases of angiomyolipoma, there was positivity for PR, and the average immunoreactivity was 0.93 (3/10 cases [30%] showed immunoreactivity ≥2; Table 3). The mean immunoreactivity for β-catenin was greater than that observed for ER-α and PR and less than that for HMB45. The difference in immunoreactivity between all 4 immunohistochemical markers was statistically significant (P < .0001; Kruskal-Wallis test).
Discussions

In this study, we showed that pulmonary LAM and renal angiomyolipomas show strong and moderate protein expression of β-catenin, respectively. Previous immunphenotypic profiling has demonstrated coexpression of the hormonal receptors ER and PR, and the melanocyte markers HMB45 and Melan-A, and muscle markers smooth muscle actin, desmin, and MyoD in pulmonary LAM. Currently, HMB45 is used as the “gold standard” to immunophenotypically identify LAM cells. In difficult biopsy specimens, HMB45 is simply a marker of perivascular epithelioid cells, whereas β-catenin can be considered a marker of proliferating cells in LAM, and is unlikely to have a key role in its pathogenesis. We demonstrated that β-catenin is highly sensitive for LAM cells with immunoreactivity superior to that of HMB45, ER-α, and PR in the majority of cases. There was no apparent difference in the staining of the 2 morphologic subtypes of LAM cells.

The specificity of β-catenin was also high; normal lung parenchyma and vascular and bronchial smooth muscle cells were negative for this marker. In addition, the agreement between 2 independent pathologists for interpreting β-catenin staining was comparable with HMB45. Our results therefore indicate that β-catenin is an additional marker of LAM cells, and, as such, may be clinically useful in the diagnostic setting.

A large percentage of renal angiomyolipomas cases demonstrated β-catenin staining in the epithelioid- and spindle-shaped smooth muscle components and the adipocyte cells. Immunoreactivity was superior to both hormone receptors, ER-α and PR, but inferior to HMB45 in the majority of cases. A possible explanation for this observed difference is that HMB45 is simply a marker of perivascular epithelioid cells, whereas β-catenin can be considered a marker of proliferative LAM cells. As such, β-catenin might be a useful adjunct marker in the diagnosis of angiomyolipoma. Moreover, these results highlight the immunphenotypic similarities between the cellular components of pulmonary LAM and renal angiomyolipoma and may indicate common genetic and biologic mechanisms underlying their pathogenesis.
LAM is a progressive disease of the lungs involving proliferation of smooth muscle cells and cystic degeneration. LAM can occur sporadically or in association with TSC through inactivating mutations in TSC1 or TSC2 with subsequent deregulation of the Rheb/mTOR/p70S6K pathway. Recent work has shed further light on its complex pathogenesis. Misexpression of the benign mesenchymal tumor gene \( \beta \)HMG2A in the adult cell population may have a central role in the pathogenesis of LAM and lead to abnormal smooth muscle proliferation in the lungs.\(^{18} \) Strong expression of cathepsin K (a papain-like cysteine protease with high matrix-degrading activity) and bcl-2 (an inhibitor of apoptosis) and an imbalance in the expression of matrix metalloproteinases and their tissue inhibitors may all lead to progressive remodeling of the lung parenchyma with resultant cyst formation.\(^{19-21} \)

Aberrant \( \beta \)-catenin signaling has been demonstrated in TSC, and up-regulation of \( \beta \)-catenin and its effectors has been demonstrated in TSC-related LAM and angiomyolipoma. The widespread influence of \( \beta \)-catenin on downstream functions such as cell proliferation, differentiation, polarity, survival, and migration could potentially account for some of the phenotypic manifestations of LAM.\(^{22} \)

Of note is that all of the cases in this study showed dysregulated expression of \( \beta \)-catenin. Work seems to indicate that the concurrent expression of mTOR effectors and \( \beta \)-catenin in LAM and angiomyolipoma may be a result of the potentiating effects of abnormal activation of both pathways, dependent on the regulatory role of TSC genes.\(^{6} \) The precise role of \( \beta \)-catenin in tumor initiation and progression, however, needs to be determined.

Currently, there are no effective treatments for LAM, and, as such, targeting \( \beta \)-catenin may represent an alternative rational treatment option made viable through recently developed small molecule antagonists of the Tcf/\( \beta \)-catenin protein complex.\(^{6} \)

Our results indicate that \( \beta \)-catenin is an excellent marker of LAM tumor cells and may be clinically useful in the diagnostic setting. We can speculate that downstream targets of \( \beta \)-catenin may account for the observed abnormalities associated with LAM.

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References


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