Frequent Estrogen and Progesterone Receptor Immunoreactivity in Renal Angiomyolipomas From Women With Pulmonary Lymphangioleiomyomatosis*

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Objective: To determine whether renal angiomyolipomas from women with pulmonary lymphangioleiomyomatosis (LAM) express estrogen receptor (ER) and progesterone receptor (PR).

Design: Retrospective study of archival tissue.

Patients: Twelve women with LAM and angiomyolipomas.

Setting: Fox Chase Cancer Center.

Interventions: ER and PR expression was studied using immunohistochemistry. The hormonal status of the patients at the time of resection of the angiomyolipoma was determined.

Results: Ten of the angiomyolipomas had ER immunoreactivity (83%), and all 12 had PR immunoreactivity (100%). The ER and PR positivity was in the smooth muscle component of the angiomyolipomas only. For five women, pulmonary LAM specimens were also available; two were ER positive (40%), and all five were PR positive (100%). All four angiomyolipomas from women receiving progesterone therapy were ER and PR positive. One tumor from a woman receiving tamoxifen was ER negative and strongly PR positive. One woman was pregnant; her tumor was ER and PR positive.

Conclusions: ER and PR expression is frequent in renal angiomyolipoma cells from women with LAM. PR was more consistently present than ER in angiomyolipomas and in LAM. Our data suggest that angiomyolipoma growth could be affected by hormonal factors. If the growth of LAM-associated angiomyolipomas slows during hormonal therapy, there are two potential implications for LAM patients: first, angiomyolipoma size could serve as a measurable indication of response to hormonal therapy; and second, surgical removal of angiomyolipomas might be avoided in some cases.

Key words: angiomyolipoma; estrogen receptor; lymphangioleiomyomatosis; progesterone receptor; smooth muscle proliferation; tuberous sclerosis complex

Abbreviations: ER = estrogen receptor; LAM = lymphangioleiomyomatosis; MoAb = monoclonal antibody; PR = progesterone receptor; TSC = tuberous sclerosis complex

Lymphangioleiomyomatosis (LAM), which affects women almost exclusively, is a rare disease of unknown etiology that was first described 60 years ago.1–5 The average age at onset of symptoms, which include shortness of breath, pneumothorax, cough, and chest pain, is 33 years.6,7 Chest radiographs typically reveal a diffuse interstitial infiltrate. Although most LAM is pulmonary, retroperitoneal and pelvic lymph node involvement can also occur.8 Most patients have a slowly declining clinical course.9 Lung transplantation is the only effective therapy for end-stage disease.

LAM can occur as an isolated disorder, which we have referred to as sporadic LAM, or in association with tuberous sclerosis (TSC). LAM affects 2.3% of individuals (or 4.6% of women) with TSC.9 TSC is an autosomal dominant disorder characterized by seizures, mental retardation, and hamartomatous tumors of the brain, heart, kidney, lung, and skin. These tumors include cerebral cortical tubers, subependymal giant cell astrocytomas, retinal hamartomas, cardiac rhabdomyomas, renal angiomyolipomas, and facial angiofibromas. Angiomyolipomas are benign tumors composed of fat, smooth muscle, and dysmorphic vessels. Renal angiomyolipomas occur in...
70% of TSC patients and in 33 to 63% of women with sporadic LAM.5,10–12 We suspect that the occurrence of angiomyolipomas in both TSC-associated and sporadic LAM reflects a common underlying genetic basis for both diseases.13

LAM consists of a diffuse proliferation of smooth muscle cells around lymphatic vessels, blood vessels, and airways. In later stages, the smooth muscle cells form nodular aggregates. Pathologically, sporadic LAM and TSC-associated LAM are indistinguishable. By electron microscopy, both types of LAM are characterized by nodular aggregates. Pathologically, sporadic and TSC-associated LAM reflect a common underlying genetic basis for both diseases.13

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The degree to which individual patients were screened for TSC has been previously reported,13 with the exception of patient 482. Patient 482 had dermatologic and ophthalmologic evaluations and brain MRI, none of which showed evidence of TSC.

The patients ranged in age from 19 to 49 years, with a mean of 33 years, at the time of resection of the angiomyolipoma. Seven of the 12 patients had the angiomyolipoma diagnosed first; 5 patients had the LAM diagnosed first. All underwent resection of the entire angiomyolipoma. All patients had lung biopsies and/or high-resolution CT scans to document the presence of pulmonary LAM. Paraffin blocks from the lung biopsy specimens were available for immunohistochemistry for five of the patients. Angiomyolipomas from four patients in the current study (patients 432, 436, 437, and 492) were previously found to have TSC2 gene loss of heterozygosity.13

**Antibodies**

A MoAb to ER α, mouse IgG1 clone ER88, raised against human recombinant estrogen receptor protein; and a MoAb to PR α, mouse IgG1 clone PR88, raised against purified human progesterone receptor protein (both from Biogenex; San Ramon, CA) were used in this study.

**Immunohistochemistry**

Five-micrometer paraffin-embedded tissue sections were deparaffinized, rehydrated, and incubated in 3% hydrogen peroxide (Fisher Scientific; Pittsburgh, PA) for 15 min to quench endogenous peroxidase activity. The sections were then incubated in Citra Solution (Biogenex), pH 6.2, at 98°C for 7 min. Slides used to analyze PR immunohistochemistry were also incubated in 0.02% trypsin for 10 min. All of the tissue sections were incubated for 20 min in 10% diluted normal horse serum (GibcoBRL; Gaithersburg, MD). Excess serum was blotted from the slides, and the sections were incubated with prediluted mouse monoclonal anti-human ER88 antibody or prediluted mouse monoclonal anti-human PR88 antibody. Phosphate-buffered saline solution was used as a control. The PR88 antibody was incubated with the slides overnight at 4°C. The ER88 antibody was incubated with the slides for 90 min at room temperature. The sections were rinsed in Optimax Solution (Biogenex) and then incubated for 30 min with the MultiLink biotinylated goat secondary antibody (Biogenex) at room temperature, followed by a rinse in phosphate buffered saline solution and incubation for 30 min with the peroxidase-conjugated streptavidin (Biogenex). After rinsing in phosphate-buffered saline solution, the slides were incubated in peroxidase substrate solution containing hydrogen peroxide and 3,3’-diaminobenzidine (Biogenex) for 2 min. Breast cancer specimens previously determined to be ER and PR positive were included as positive controls with each reaction.

The intensity of the staining was graded as follows: negative (−), weak (+), moderate (+ +), strong (+ + +), or very strong (+ + + +). The staining intensity of each slide was determined by comparing it to the intensity of an ER- and PR-positive breast cancer specimen that was assayed simultaneously. The staining intensity of the most positive nuclei in the angiomyolipoma was compared with the intensity of the most positive nuclei in the breast cancer. The intensity of the breast cancer nuclei was designated as strong (++) for the purpose of this comparison. Two angiomyolipoma specimens (443 and 482) had nuclear staining for PR that was more intense than the breast cancer control and were designated as very strong (++ + + +). The percentage of positive nuclei was also estimated for each specimen.

**RESULTS**

As shown in Table 1, all 12 of the angiomyolipomas were PR positive (100%) and 10 were ER positive.
positive (83%). The degree of positivity for ER, relative to the breast cancer specimen used as a positive control, was moderate in four cases (33%), weak in six cases (50%), and absent in two cases (17%). The PR positivity was strong in five cases (42%), moderate in six cases (50%), and weak in one case (8%). The ER positivity was seen in between 0% and 50% of the nuclei. The PR positivity was seen in 20 to 80% of the smooth muscle cell nuclei. Of the five patients for whom lung specimens were also available, two patients (40%) were ER positive and five patients (100%) were PR positive. The smooth muscle cells of all five of the pulmonary LAM specimens had PR expression. Two specimens (40%) also had ER expression.

Table 1—ER and PR Immunoreactivity in Angiomyolipomas and Pulmonary LAM

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hormonal Status†</th>
<th>AML</th>
<th>LAM</th>
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<tr>
<td></td>
<td></td>
<td>ER</td>
<td>PR</td>
</tr>
<tr>
<td>432</td>
<td>–</td>
<td>+ (+30)</td>
<td>+ (+30)</td>
</tr>
<tr>
<td>436</td>
<td>–</td>
<td>+ (+30)</td>
<td>+ (+30)</td>
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<tr>
<td>437</td>
<td>Progesterone</td>
<td>+ (20)</td>
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</tr>
<tr>
<td>443</td>
<td>Pregnant 10 wk</td>
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<td>+ (+50)</td>
</tr>
<tr>
<td>480</td>
<td>Progesterone</td>
<td>+ (+50)</td>
<td>+ (+50)</td>
</tr>
<tr>
<td>481</td>
<td>Progesterone</td>
<td>+ (30)</td>
<td>+ (80)</td>
</tr>
<tr>
<td>482</td>
<td>Tamoxifen</td>
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<td>+ (+40)</td>
</tr>
<tr>
<td>487</td>
<td>–</td>
<td>+ (30)</td>
<td>+ (30)</td>
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<td>489</td>
<td>–</td>
<td>–</td>
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<tr>
<td>491</td>
<td>–</td>
<td>+ (+30)</td>
<td>+ (+30)</td>
</tr>
<tr>
<td>482</td>
<td>–</td>
<td>+ (10)</td>
<td>+ (20)</td>
</tr>
<tr>
<td>N = 12‡</td>
<td></td>
<td>10 (83)</td>
<td>12 (100)</td>
</tr>
</tbody>
</table>

*Data are presented as degree of positivity (percentage of positive cells), unless otherwise indicated; + = weak; + + = moderate; + + + = strong; + + + + = very strong; – = negative; AML = angiomyolipoma.
†Hormonal status at the time of resection of the renal angiomyolipoma. A dash indicates that the patient was not pregnant and not receiving hormonal therapy. None of the patients whose lung tissue was studied were receiving hormonal therapy at the time of the lung biopsy.
‡Number (percentage) of the AML and LAM specimens that stained positively for ER or PR.

Because LAM has been reported to clinically worsen during pregnancy, and is often treated with hormonal agents, we determined the hormonal status of the women in this study at the time of the removal of the angiomyolipoma (Table 1). At the time of lung biopsy, none of the five women from whom lung biopsy specimens were obtained was receiving hormonal therapy. At the time of the angiomyolipoma surgery, one patient was pregnant, four were receiving progesterone therapy, and one was receiving tamoxifen therapy. The angiomyolipoma from the patient who was receiving tamoxifen therapy (patient 482) was ER negative and very strongly PR positive. PR and ER positivity were seen in the angiomyolipomas from all four women receiving progesterone therapy (patients 437, 480, 481, and 490), as well as in the tumor from the woman who was pregnant (patient 443).

**Discussion**

LAM occurs almost exclusively in women of childbearing age. The underlying reasons for this are not known. A better understanding of the hormone receptor status in LAM could contribute toward the rational selection of appropriate hormonal therapy. Currently, many women with LAM are treated with high-dose progesterone, oophorectomy, or tamoxifen. The clinical efficacy of these therapies has never been assessed in a controlled study.

In this study, we examined ER and PR expression in angiomyolipomas from 12 women with sporadic LAM and in pulmonary LAM specimens from 5 women. We found PR immunoreactivity in the smooth muscle component of all 12 of the angiomyolipomas (100%). ER immunoreactivity was present in 10 of the tumors (83%). The smooth muscle cells of all five of the pulmonary LAM specimens had PR expression. Two specimens (40%) also had ER expression.

The strongest PR immunoreactivity in an angiomyolipoma was seen in patients 443 and 482. This strong positivity may be related to hormonal factors: patient 443 was in the first trimester of pregnancy,
and patient 482 had been on tamoxifen therapy for 10 years at the time that the angiomyolipoma was removed. The relationship between tamoxifen therapy and changes in ER and PR in breast cancer is not well defined. In a recent immunohistochemical study of primary breast tumors during tamoxifen therapy, a decrease in ER was seen in 3 of 15 tumors, and an increase in PR was seen in 7 of 17 tumors. Tumors that responded to the tamoxifen therapy were more likely than nonresponding tumors to have a decrease in ER and an increase in PR. The relationship between progesterone therapy and changes in ER and PR in primary tumors is not known. Four patients in our study were receiving progesterone therapy at the time the angiomyolipoma was removed. All four had weak or moderate ER positivity and moderate or strong PR positivity in the angiomyolipomas.

This is, to our knowledge, the first series of LAM-associated angiomyolipomas that has been studied for ER and PR expression. There is one other study by Tawfik et al that examined a single angiomyolipoma from a woman with sporadic LAM for ER and PR immunoreactivity. This patient was 25 weeks pregnant at the time of angiomyolipoma resection. The angiomyolipoma was negative for both ER and PR.

Pulmonary LAM samples were available for immunohistochemistry for five of the patients in our study, making this one of the largest series of pulmonary LAM specimens studied for ER and PR immunoreactivity. All five of our pulmonary specimens were immunoreactive for PR in the pulmonary LAM, and two were immunoreactive for ER. The results of our study and previous studies in which ER and PR immunoreactivity has been studied in LAM are summarized in Table 2. Ohori et al reported five cases of pulmonary LAM, none of which had ER or PR immunoreactivity. Kinoshita et al reported two cases of pulmonary LAM, both of which were ER positive and PR negative. Berger et al reported another two cases: one was ER and PR positive, and one was ER positive and PR negative. Other reports are of single patients, including one who was ER negative, with 15% of the nuclei PR positive, and one with strong staining for PR and weak staining for ER in > 80% of the smooth muscle cell nuclei. There is only one previous report in which both lung and angiomyolipoma cells from the same patient were examined: the pregnant patient studied by...
Pulmonary LAM cells from this patient had weak immunoreactivity for ER and no immunoreactivity for PR.21

The clinical and genetic distinctions between LAM and TSC have been debated.31 Chromosome 16p13 loss of heterozygosity in the region of the TSC2 gene has been found in angiomyolipomas from women with LAM,13 suggesting that these angiomyolipomas may result from mutations in both copies of the TSC2 gene. We have previously found that 55% of TSC-associated angiomyolipomas are PR positive.25 Among angiomyolipomas from patients who do not have either TSC or LAM, PR immunoreactivity was present in only 7%.25 The PR positivity in the LAM-associated angiomyolipomas could be a further indication of common genetic and biological mechanisms underlying TSC and sporadic LAM. However, in our previous study of TSC angiomyolipomas, none were ER positive,25 while 83% of the LAM angiomyolipomas were ER positive. This could indicate a true biological difference between the expression of ER and PR in angiomyolipomas from women with TSC vs those with sporadic LAM. Alternatively, it is possible that angiomyolipomas from women who develop LAM (whether or not they have TSC) are more likely to be ER immunoreactive than angiomyolipomas from women who do not develop LAM. The TSC patients in our previous study were not ascertained for the presence or absence of LAM. However, it is important to note that these two studies used different ER antibodies (a Biogenex MoAb in the current study and a Dako [Carpinteria, CA] MoAb in the previous study).

There are currently no in vitro or in vivo data to support the hypothesis that the proliferation of LAM cells is dependent on hormonal stimuli. There is, however, considerable indirect evidence that smooth muscle cell growth in pulmonary LAM is influenced by steroid hormones. This evidence includes the following: the occurrence of LAM almost exclusively in women; the presence of ER and PR in pulmonary LAM cells19,22,24,28; the reports of exacerbation of LAM during pregnancy21,32,33 and during estrogen therapy34; and the apparent therapeutic response to exogenous hormonal agents in some patients, as reviewed by Kalassian et al2 and Sullivan.3 Our data indicate that the smooth muscle cells in angiomyolipomas may also be capable of responding to hormonal stimuli.

Clinical improvement or stabilization of pulmonary symptoms during hormonal therapy for LAM has occurred in many cases and has been recently reviewed by Sullivan.3 There are no randomized or controlled studies of LAM progression during hormonal therapy. Such studies are likely to be challenging to perform because of the difficulty in quantitating disease progression. Our data suggest that angiomyolipomas, like pulmonary LAM, may be responsive to hormonal therapy. It is possible, therefore, that in women with sporadic LAM who have angiomyolipomas, the size of the angiomyolipoma could be used as a marker of clinical response to hormonal therapy. The size of angiomyolipomas can be objectively determined, and many angiomyolipomas have measurable growth in 1 year.35–37 There are currently no reports of the growth rates of angiomyolipomas during hormonal therapy for LAM.

In summary, we studied 12 angiomyolipomas from women with sporadic LAM and found that 10 were ER immunoreactive and all 12 were PR immunoreactive. Of five specimens of pulmonary LAM, we found that two were ER immunoreactive and all five were PR immunoreactive. It is important to note that the antibody used in this study was raised against the estrogen receptor α. Whether estrogen receptor β is present in either pulmonary LAM cells or angiomyolipoma cells is not known. Elucidation of the role of steroid hormone receptors in the pathogenesis of LAM could contribute to both our understanding of

**Table 2—Literature Review of ER and PR Immunoreactivity in LAM-Associated Angiomyolipomas and Pulmonary LAM**

<table>
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<tr>
<th>First Author</th>
<th>Year</th>
<th>AML</th>
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<th>PR</th>
<th>LAM</th>
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<td>Colley30</td>
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<td>Ohori28</td>
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<td>Kinoshita29</td>
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<td>0/2</td>
<td></td>
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<tr>
<td>Tawfik21</td>
<td>1996</td>
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<tr>
<td>Logginidou</td>
<td>This report</td>
<td>10/12</td>
<td>12/12</td>
<td>2/5</td>
<td>5/5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total†</td>
<td></td>
<td>10/13 (77)</td>
<td>12/13 (92)</td>
<td>8/17 (47)</td>
<td>8/17 (47)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data are presented as No. of positive specimens/No. tested, unless otherwise indicated; see Table 1 for abbreviation.
†Data are presented as No. of positive specimens/No. tested (percent of total tested).

Tawfik et al.21 Pulmonary LAM cells from this patient had weak immunoreactivity for ER and no immunoreactivity for PR.21
the hormonal factors that contribute to smooth muscle proliferation in LAM, and also to the selection of appropriate hormonal therapy for women with LAM.

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REFERENCES