Estrogen Enhances whereas Tamoxifen Retards Development of Tsc Mouse Liver Hemangioma: A Tumor Related to Renal Angiomyolipoma and Pulmonary Lymphangioleiomyomatosis

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Abstract

Pulmonary lymphangioleiomyomatosis and abdominal angiomyolipoma are related lesions for which there is no authentic animal model. Both of these proliferative lesions occur in sporadic patients, and at much higher frequency in patients with tuberous sclerosis, which is due to mutations in the TSC1 and TSC2 genes. Tsc1+/− and Tsc2−/− mice frequently develop liver hemangioma. We found that the Tsc mouse liver hemangioma are composed predominantly of endothelial cells but with a smooth muscle component, and express HMB45 antigen, estrogen receptor, and progesterone receptor, similar to lymphangioleiomyomatosis and angiomyolipoma. Estrogen treatment significantly accelerated the development of liver hemangioma in Tsc1−/− female mice, with 91% having liver hemangioma and 55% having severe lesions by 7 months of age. Similarly, an increased frequency and severity of liver hemangiomas was seen in Tsc1−/− males treated with estrogen. In contrast, tamoxifen treatment for 9 months significantly reduced the frequency and severity of hemangiomas in Tsc1−/− female mice. In addition, estrogen treatment significantly increased serum vascular endothelial growth factor levels in Tsc1−/− mice, whereas tamoxifen reduced vascular endothelial growth factor levels. These mouse model observations indicate the importance of estrogen signaling in vivo for the growth of tuberous sclerosis lesions, suggesting the possible benefits of tamoxifen therapy for the treatment of angiomyolipoma and lymphangioleiomyomatosis. (Cancer Res 2005; 65(6): 2474-81)

Introduction

Pulmonary lymphangioleiomyomatosis is a rare nonneoplastic lung disease with an incidence of ~1/1,000,000 in the United States and Europe (1). It occurs almost exclusively in women during their reproductive years or while receiving estrogen replacement therapy, and leads to progressive respiratory failure (2, 3). Histologically, there are two main components to lymphangioleiomyomatosis pathology. Smooth muscle cell proliferation is invariably seen within the lung parenchyma, with cells varying from small round or oval cells, to small to medium spindle-shaped cells, to large epithelioid cells (2–4). Despite this variable morphology, all of these cells express smooth muscle actin, and are thought to be part of a continuous morphologic spectrum. Second, there is progressive destruction of pulmonary connective tissue with the formation of cysts that are diffusely distributed throughout the lung (2–4).

Up to 63% of women with lymphangioleiomyomatosis also have renal angiomyolipoma (3, 5). Angiomyolipomas are benign tumors consisting of smooth muscle cells, fibrous tissue, adipose tissue, and abnormally formed vascular channels (6). Like lymphangioleiomyomatosis, angiomyolipomas are more common in females than in males with a lifetime incidence of 1 in 330 women and 1 in 5,000 men (7). In addition, the occasional improvement of clinical symptoms of lymphangioleiomyomatosis by oophorectomy, progesterone, or tamoxifen (3, 8), the detection of estrogen/progesterone receptors in lymphangioleiomyomatosis tissues (4), and the development or worsening of lymphangioleiomyomatosis during pregnancy or exogenous estrogen therapy (3), all suggest that female sex hormones have a role in the pathogenesis of lymphangioleiomyomatosis. Both angiomyolipomas and pulmonary lymphangioleiomyomatosis occur sporadically and in association with tuberous sclerosis (TSC).

TSC is an autosomal dominant disorder characterized by multigorgan hamartomatosis as a result of mutations in either TSC1 or TSC2 (6). TSC follows the Knudsen model of tumor suppressor gene function in that TSC lesions often show loss of heterozygosity for the remaining normal allele of either TSC1 or TSC2 (9, 10). Mutations in TSC2 are much more common than in TSC1, and are associated with more severe clinical features (11). Angiomyolipomas occur in the kidney, liver, or both in about 80% of adult TSC patients and are more common in female compared with male patients (12, 13). Clinically significant pulmonary lymphangioleiomyomatosis occurs in about 5% of adult TSC female patients, including patients with each of TSC1 and TSC2 mutations, and is the third leading cause of death (6, 8). Subclinical involvement is seen in about 50% of adult TSC women (14, 15). Loss of heterozygosity for TSC2 has been seen in both sporadic and TSC-associated angiomyolipoma (1, 9, 10, 16, 17), and proliferative smooth muscle cells from lymphangioleiomyomatosis lesions of sporadic patients often have point mutations in one of the two alleles of TSC2 (18). Pathologically, there are no major differences between sporadic and TSC-associated lymphangioleiomyomatosis and angiomyolipoma.

Beyond the involvement of the TSC1 and TSC2 genes, there is limited understanding of the pathogenesis of lymphangioleiomyomatosis and angiomyolipoma, attributable in part to the lack of an in vivo model of the disease. Our laboratory has described Tsc mouse models that have been developed by gene targeting

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(19, 20). Both Tsc1+/− and Tsc2+/− mice develop liver hemangiomas, consisting of proliferative smooth muscle cells, endothelial cells, and vascular channels (Fig. 1) at high frequency (19, 20). Hemangiomas occur at significantly higher frequency and severity, and cause higher mortality in female (92% incidence, 45% mortality), compared with male (67% incidence, 10% mortality) Tsc1+/− mice (20). These data suggest that estrogen may contribute to development of hemangiomas in these Tsc mouse models, similar to angiomyolipomas and lymphangioleiomyomatosis. In this study, we examined the histologic and expression characteristics of Tsc mouse liver hemangiomas, and assessed the in vivo responses of these tumors to estrogen and tamoxifen treatment.

Materials and Methods

Mouse Studies. All results reported here were obtained through analysis of Tsc1+/− mice in the 129/Sv strain (20). Wild-type controls and Tsc1+/− mice were generated from the same breeding colony. All procedures were carried out in accordance with the Guide for the Humane Use and Care of Laboratory Animals, and the study was approved by the Harvard Medical Area Standing Committee on Animals. Mice were euthanized when weight loss of 10%, reduced movement, or other signs of morbidity were seen.

17β-Estradiol (E2) and tamoxifen were given to mice using sustained-release pellets designed to last 90 days (Innovative Research, Sarasota, FL). Five and 15 mg E2 pellets and 15 mg tamoxifen pellets were implanted s.c. in the interscapular area. Repeat doses, when used, were given at 90-day intervals. All mice were observed at least twice weekly.

Necropsy analysis included examination of the liver and kidneys and other organ systems. Liver hemangioma were graded from 0 to 5 by a single observer (V. Walker) who was blinded to genotype and treatment status: 0, no gross or microscopic lesion; 1, microscopic tumor only; 2, gross hemangioma in one lobe; 3, gross hemangioma in two lobes of the liver; 4, extensive hemangioma in multiple lobes; and 5, a mouse found to have died from hemorrhage from a liver hemangioma. Hemangiomas graded in the 3 to 5 range were considered severe. The kidney severity score for kidney cystadenomas was determined as a summed score for all lesions in a kidney, scoring each individual tumor grossly as follows: 1 for tumors <1 mm; 2 for 1 to 2

Figure 1. Histology of Tsc mouse liver hemangiomas. A and B, representative sections of Tsc1+/− mouse liver hemangioma stained with H&E. Variable sized vascular channels are seen with a predominance of spindle-shaped cells, including irregular endothelial cells lining the vascular channels. C, immunohistochemistry of hemangioma showing a predominance of CD31-positive endothelial cells in the lesion. D, a gross picture of liver hemangioma (grade 4) from a Tsc1+/− female treated with E2 (cohort 1). Note the dark region due to hemorrhage. E, H&E section of liver hemangioma of the same mouse in (D); F, immunohistochemistry of liver hemangioma showing ER expression in smooth muscle and to a lesser extent in endothelial cells.
1.5 mm; 3 for 1.5 to 2 mm; 5 for 2 to 3 mm; 10 for 3 to 4 mm. All kidneys were cut transversely into five pieces, and microscopic examination was done on each. Microscopic examination of the liver was done on a single H&E-stained section covering the entire liver.

**Immunoblot Analysis.** Immunoblot analysis was done on mouse liver lesions, normal liver tissues, Tsc1+/- and Tsc2+/- mouse embryonic fibroblasts (20), and on angiomylipomas from a postmortem kidney from a TSC patient with germ line mutation TSC2 E293XinsC, as described (21, 22). Antibodies used were: HMB45 (NeoMarkers, Fremont CA); estrogen receptor (ER), progesterone receptor (PR), actin, and S6K (Santa Cruz Biotechnology, Santa Cruz CA). Membranes were developed with horseradish peroxidase-conjugated anti-mouse or anti-rabbit secondary antibodies (Santa Cruz Biotechnology) using enhanced chemiluminescence (Pierce, Rockford, IL). All immunoblots shown in one row of a figure are from the same gel blot exposure.

**Vascular Endothelial Growth Factor Analysis.** Serum vascular endothelial growth factor (VEGF) measurements were determined by ELISA kit specific for mouse VEGF (Oncogene Research Products, San Diego, CA), as described previously (22).

**Survival Curves and Statistical Analysis.** Kaplan-Meier cumulative survival plots were calculated using Statview v 5.0 and comparisons made using the log-rank (Mantel-Cox) test. The Fisher exact test was used for analysis of 2 × 2 tables comparing observations between different cohorts of mice. Sets of observations for different cohorts of mice (e.g., percentage of liver involvement by hemangioma) were compared using the t test.

**Results**

**Characteristics of Tsc1+/- and Tsc2+/- Mouse Liver Hemangioma.** Tsc1+/- and Tsc2+/- mouse liver hemangioma are characterized by extensive abnormally organized spindle-shaped CD31-positive endothelial cells, that form aberrant highly variable vascular channels (Fig. 1A–C). In some regions, the endothelial cell proliferation is quite dense with tiny vascular spaces, although in other regions, relatively large thin-walled (maximum, 2.5 mm) vascular channels are seen. CD31-negative smooth muscle cells were also seen in these lesions to a smaller extent.

A major diagnostic feature of lymphangioleiomyomatosis and angiomylipomas is their immunoreactivity with HMB45 antibody (23). Western blot analysis using HMB45 showed variable expression of the antigen in different hemangiomas from Tsc1+/- and Tsc2+/- mice (Fig. 2A), and was similar to that seen in angiomylipoma samples, as reported previously (23, 24). Due to antibody reactivity limitations and despite considerable effort, we could not directly show HMB45 staining on mouse liver hemangioma, in contrast to human angiomylipoma tissue sections (data not shown). The reactivity of HMB45 by immunoblot was observed only in liver hemangiomas, but not in either mouse embryonic fibroblast cells lines lacking either Tsc1 or Tsc2, or in Tsc1+/- and Tsc2+/- kidney cystadenomas (Fig. 2B). These observations suggest that Tsc1+/- and Tsc2+/- mouse liver hemangioma is a lesion related to human lymphangioleiomyomatosis and angiomylipoma.

**Female Sex Hormone Receptor Expression in Hemangiomas.** Expression of ERα and PR were observed in liver hemangioma sections by immunohistochemistry and immunoblot analyses. ERα positivity was seen in both smooth muscle cells and endothelial cells in hemangioma (Fig. 1F). PR positivity was also present in both cell types (data not shown). Immunoblot analysis confirmed that ER and PR were expressed in hemangiomas similar to normal liver and kidney tissues, whereas PR expression in liver hemangiomas was higher than in normal liver and normal kidney, whereas ER expression was similar among these tissues. E, ER and PR expression in liver hemangiomas from both Tsc1+/- and Tsc2+/-; Tsc1+/- and Tsc2+/- mouse embryonic fibroblasts (MEFs) and MCF7 cells.
angioymolipomas (25) (Fig. 2C, D, and E). ERα expression was specific to hemangiomas, whereas PR expression was more variable and seen in normal liver (Fig. 2C). Similarly, ERα expression was higher in TSC2-associated angioymolipoma than normal kidney, although PR expression was similar in angioymolipomas and normal kidney (Fig. 2D). These observations provide further evidence of the similarity of Tsc mouse liver hemangiomas to human angioymolipomas and lymphangioleiomyomatosis.

**Effects of Estrogen and Tamoxifen Treatment on Hemangioma Growth.** To explore the effects of estrogen and tamoxifen treatment on liver hemangioma growth, we prepared several cohorts of Tsc1+/− and control mice (Table 1). We studied Tsc1+/− mice and not Tsc2+/− mice because the Tsc1− allele has been maintained in the 129/sV strain in which the frequency and severity of liver hemangioma is higher than in other strains.1 Three female cohorts (1, 3, and 5) received E2 beginning at age 8 weeks, and were initially planned to receive estrogen therapy for 1 year. However, severe toxicity occurred in these mice, such that the third (E2, 5 mg), and the second and third (E2, 15 mg) doses were withheld. These mice had a median survival of 7.5 months (groups 1 and 3) and 9.5 months (group 5), and all had died by the age of 11 months regardless of Tsc1 genotype. The cause of death in most of these mice was renal failure due to tubular necrosis, fibrosis, and hydronephrosis. Two Tsc1+/− mice, one each in cohorts 1 and 3, had a large amount of blood in the peritoneal cavity at necropsy, which was due to massive bleeding from a liver hemangioma.

As reported previously (20), we observed that the severity and mortality of liver hemangioma in untreated Tsc1+/− females was greater than that in untreated Tsc1+/− males (cohorts 6 and 8, \( P = 0.0152 \); Table 1). Liver hemangioma frequency was similar in

### Table 1. Cohorts of Tsc1+/− and control mice with and without treatment, survival, and liver hemangioma incidence and severity

<table>
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<th>Sex</th>
<th>Treatment</th>
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<th>Age started (months)</th>
<th>Planned end point (months)</th>
<th>Median survival (months)</th>
<th>Percentage dying prematurely</th>
<th>No. analyzed*</th>
<th>Frequency of hemangioma</th>
<th>Frequency of severe hemangioma</th>
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<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
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<td>F</td>
<td>±</td>
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<td>2</td>
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<td>9</td>
<td>9 (100%)</td>
<td>8 (89%)</td>
<td>42</td>
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*Note that not all mice that died early could be analyzed pathologically due to decomposition of tissues and/or cannibalism by cage mates.


NOTE: Repeated doses were given at 90-day intervals.

Abbreviations: F, female; M, male; Tam, tamoxifen.
hemangioma was significantly higher in Tsc1+/- females compared with Tsc1+/- males (9 of 11 versus 1 of 11, \( P = 0.0019 \); Fig. 3A; Table 1). In contrast, Tsc1+/- male mice treated with a single dose of E2 at 5 mg (cohort 9, Table 1), developed liver hemangioma at a rate similar to that of untreated Tsc1+/- females, with 2 of 11 deaths prior to age 14 months (Fig. 3A), and severe liver hemangiomas in 8 of 9 (Fig. 3B; Table 1). We also determined the extent of liver replacement by hemangioma microscopically (Table 1, far right). The extent of involvement of the liver by hemangioma was significantly higher in Tsc1+/- females (cohort 6) compared with Tsc1+/- males (cohort 8; 56% versus 15%, \( P = 0.005 \); Table 1). Similarly, E2 treatment of Tsc1+/- males (cohort 9) significantly increased the extent of hemangioma (from 15% to 42%, \( P = 0.004 \); Table 1).

We also examined the effect of tamoxifen on hemangioma development, both in a preventive mode, with three doses given over 9 months (cohort 4, Table 1), and in a treatment mode, a single dose given at age 10 months (cohort 7, Table 1). In cohort 4, there were no premature deaths in contrast to the untreated cohort 6 (\( P = 0.0216 \), Fisher exact test). In addition, prolonged tamoxifen treatment significantly reduced both the frequency of hemangioma, the frequency of severe hemangioma (Fig. 3C), and the extent of involvement of the liver (\( P = 0.020, 0.0017, 0.034 \), respectively; Table 1). Although single-dose tamoxifen treatment had no significant effect on survival or liver hemangioma frequency or severity (Table 1), the extent of hemangioma involvement in the liver was significantly less in cohort 7 in comparison to untreated Tsc1+/- cohort 6 females (\( P = 0.04 \); Table 1).

E2 Increases VEGF Production and Vascularization. Recently, we have shown that deficiency in either Tsc1 or Tsc2 results in a marked increase in VEGF production \textit{in vitro} and \textit{in vivo}, and correlates with the extent of tumor formation in Tsc mice (22). Therefore, we examined the effect of E2 and tamoxifen treatment on serum levels of VEGF in these cohorts (Table 2). VEGF levels in wild-type males and females of age 17 to 19 months were all <80 pg/mL, significantly less than levels seen in Tsc1+/- males and females of age 13 to 14 months (Fig. 4A). VEGF levels were significantly higher in Tsc1+/- females in comparison to Tsc1+/- males (\( P = 0.047 \); Fig. 4A), correlating with the extent of liver hemangioma and kidney cystadenomas in these mice. In addition, serum VEGF levels in the tamoxifen 9-month treatment cohort of Tsc1+/- females (cohort 4) were significantly lower than levels seen in untreated Tsc1+/- females in comparison to untreated Tsc1+/- males (\( P = 0.047 \); Fig. 4B; Table 2). In contrast, VEGF levels were very high in the E2-treated Tsc1+/- females in cohort 1, higher than was seen in much older untreated Tsc1+/- females (cohort 6) of the same age (\( P = 0.047 \); Fig. 4B; Table 2). These observations confirm that VEGF is strongly associated with hemangioma development in Tsc mouse models.

Kidney Cystadenomas. Tsc1+/- mice develop kidney lesions, which vary from pure cysts with cuboidal lining cells, to cysts with papillary projections, to solid adenomas. We observed that renal cystadenomas develop at higher frequency and severity in Tsc1+/- untreated females in comparison to untreated Tsc1+/- males, \( P = 0.0431 \) (Fig. 5). Tamoxifen treatment for 9 months significantly reduced the frequency (\( P = 0.045 \)) and severity (\( P = 0.005 \)) of renal lesions in Tsc1+/- females (cohort 4) in comparison to untreated Tsc1+/-

![Figure 3. Survival and liver hemangioma frequency in Tsc1+/--treated mice. A, Kaplan-Meier cumulative survival plot for untreated and estrogen-treated Tsc1+/- males. B and C, frequency of liver hemangioma and severe liver hemangioma in Tsc1+/- mice treated with either E2 or tamoxifen; B, estrogen treatment increased the frequency of severe liver hemangiomas in Tsc1+/- males to a level similar to that of untreated Tsc1+/- females, much higher than seen in untreated Tsc1+/- males; C, 9-month tamoxifen treatment reduced the frequency of liver hemangioma and severe liver hemangioma in Tsc1+/- females; *, **, pairwise statistical comparisons (Fisher exact test) between different groups.](image-url)
females of the same age (cohort 6; Fig. 5). *Tsc1+/−/C0* females treated with a single tamoxifen dose (cohort 7) had a lower kidney severity score than untreated *Tsc1+/−/C0* females (Fig. 5), but the difference did not achieve statistical significance. Renal cystadenoma development could not be assessed in the E2-treated *Tsc1+/−/C0* females (cohorts 1 and 3) due to the extensive renal pathology induced by estrogen treatment. Estrogen treatment seemed to reduce the severity of kidney cystadenomas in *Tsc1+/−/C0* males in comparison to untreated *Tsc1+/−/C0* males (*P* = 0.044; Fig. 5).

**Discussion**

*TSC1* and *TSC2* have been established as having a critical role in the pathogenesis of both angiomyolipomas and lymphangioleiomyomatosis. Angiomyolipomas occur at high frequency (~80%) in patients with TSC (6, 12, 13), and these lesions typically show loss of the remaining normal allele of either *TSC1* or *TSC2* consistent with a two-hit model of disease development (1, 9, 10, 16, 17). Adult female TSC patients have a 5% incidence of clinically significant lymphangioleiomyomatosis (6, 8), and radiographic evidence of the condition is found in about 50% when high-resolution chest computed tomography scans are done (14, 15). Both angiomyolipoma and lymphangioleiomyomatosis are rare in the general, non-TSC population. However, the *TSC2* gene has a critical role in the pathogenesis of these lesions in non-TSC patients, with both point mutations and loss of heterozygosity being seen (1, 18).

Clinical observations on the increased frequency and severity of both angiomyolipomas and lymphangioleiomyomatosis in females (2, 3, 7) have suggested that female sex hormones have a critical role in fostering the development of these lesions. This is particularly true for lymphangioleiomyomatosis, which occurs nearly exclusively in females, typically has onset following puberty, and can worsen during pregnancy (3). However, clinical experience with hormonal therapy or antiestrogen treatment of lymphangioleiomyomatosis has been uneven, with some dramatic anecdotal responses, but many other treated patients in which there seemed to be no benefit (3, 8, 26). Systematic study of female hormone sensitivity in an authentic animal model of lymphangioleiomyomatosis has a high priority.

In addition to histologic features, lymphangioleiomyomatosis and angiomyolipoma cells are characterized by their reactivity with mouse monoclonal antibody HMB45, which was originally generated against an extract of human melanoma cells (23, 24). The HMB45 antibody reacts with variably sized proteins of the melanocyte-lineage gene SILV/PMEL17/GP100 (27). In lymphangioleiomyomatosis and angiomyolipoma cells, the binding sites for HMB45 antibody are cytoplasmic granules that resemble immature melanosomes (23, 24). The reason for the occurrence of these HMB45 organelles in lymphangioleiomyomatosis and angiomyolipoma cells is unknown.

Our observation that *Tsc* mouse liver hemangioma expresses the HMB45 antigen suggests that these liver hemangiomas are related to patient angiomyolipomas and lymphangioleiomyomatosis. The expression of both ER and PR by liver hemangioma is also similar to angiomyolipoma and lymphangioleiomyomatosis, providing further evidence for this similarity. These observations also fit with

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*Grade of liver hemangioma severity (see Materials and Methods).*

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Table 2. Serum VEGF levels in *Tsc1+/−* female mice treated with or without estrogen or tamoxifen

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Research.
the observations on the sex differences in the incidence and severity of the hemangiomas.

We have shown that E2 treatment increased liver hemangioma frequency and severity in Tsc1+/− females (Table 1), and increased the severity of liver hemangiomas in Tsc1+/− male mice (Fig. 3B; Table 1). Although tamoxifen treatment did not completely prevent hemangioma development in Tsc1+/− females, it did significantly reduce both their incidence and severity (Fig. 3C; Table 1). The observations are consistent with a model in which female sex hormones are not absolutely critical for development of liver hemangioma but can significantly affect the growth and development of these lesions.

E2 is known to promote angiogenesis activity in vitro and in vivo (29, 30). It has been shown that E2 stimulates VEGF expression in the uterus (28), and in vascular smooth muscle cells (31). Furthermore, it has been reported that E2 treatment increases tumor extracellular levels of VEGF in estrogen-dependent breast cancer models (32), whereas tamoxifen decreases it (33, 34). Cells lacking Tsc1 or Tsc2 have enhanced production of VEGF through both mTOR-dependent and -independent mechanisms (22, 35). We have observed that E2 treatment significantly increased serum VEGF levels in Tsc1+/− females. In our review of the pathology, liver hemangioma from the E2-treated Tsc1+/− females also seemed to contain larger vascular channels compared with untreated Tsc1+/− females. The observations suggest that estrogen-enhanced VEGF production may partially account for sex differences in severity in Tsc1+/− mice as well as the effects of estrogen and tamoxifen treatment. However, it is also possible that the increased VEGF levels seen in estrogen-treated mice simply reflect the larger tumors in those animals.

In summary, the observations presented here provide evidence that Tsc mouse liver hemangiomas are pathologic lesions whose pathogenesis and natural history are similar to those of angiomylipoma and lymphangioleiomyomatosis which develop in both TSC and sporadic patients. Although not absolutely required, estrogen accelerates whereas tamoxifen retards development of hemangiomas in Tsc1+/− mice. The observations support continuing study of the benefits of female sex hormone intervention in the treatment of angiomylipoma and lymphangioleiomyomatosis.

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Figure 5. Severity of kidney cystadenomas in Tsc1−/− mice. The kidney severity score, a measure of the involvement of the kidney by cystadenoma (see Materials and Methods), is shown for five different cohorts of Tsc1−/− mice. Note that the kidney severity score is higher in untreated Tsc1−/− females in comparison to untreated Tsc1−/− males. Nine-month tamoxifen treatment significantly reduced the kidney score in Tsc1−/− females, whereas 3-month tamoxifen treatment had a similar but smaller effect. Estrogen treatment reduced the kidney severity score in Tsc1−/− males. * pairwise statistical comparisons (Fisher exact test) between different groups.

Figure 4. Estrogen increases VEGF levels and vascularization. A, comparison of serum VEGF levels in wild-type and Tsc1−/− mice. Note that VEGF levels are much higher in Tsc1−/− females than Tsc1−/− males of the same age, and both are higher than control mice of either sex. B, comparison of VEGF levels among E2- and tamoxifen-treated Tsc1−/− mice. Note that VEGF levels were much higher in E2-treated mice than controls, and were lower in tamoxifen-treated mice than controls. *, pairwise statistical comparisons (Fisher exact test) between different groups.
References


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