Renal Angiomyolipomas from Patients with Sporadic Lymphangiomyomatosis Contain Both Neoplastic and Non-Neoplastic Vascular Structures

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Renal angiomyolipomas are highly vascular tumors that occur sporadically, in women with pulmonary lymphangiomyomatosis (LAM), and in tuberous sclerosis complex (TSC). The goal of this study was to determine whether the distinctive vessels of angiomyolipomas are neoplastic or reactive. We studied angiomyolipomas with loss of heterozygosity (LOH) in the *TSC2* region of chromosome 16p13 from patients with LAM. We found that angiomyolipomas contain five morphologically distinct vessel types: cellular, collagenous, hemangiopericytic, glomeruloid, and aneurysmatic. Using laser capture microdissection, we determined that four of the vessel types have *TSC2* LOH and are therefore neoplastic. One vessel type, collagenous vessels, did not have LOH, and is presumably reactive. Recently, activation of S6 Kinase and its target S6 ribosomal protein (S6) was demonstrated in cells lacking *TSC2* expression. We found that angiomyolipoma vessel types in which LOH were detected were immunoreactive with anti-phospho-S6 antibodies. Angiomyolipoma cells without LOH, including the endothelial component of the vessels, were not immunoreactive. To our knowledge, angiomyolipomas are the first benign vascular tumor in which the vascular cells, rather than the stromal cells, have been found to be neoplastic. Angiomyolipomas appear to reflect novel vascular mechanisms that may be the result of activation of cellular pathways involving S6 Kinase. (*Am J Pathol* 2003, 162:491–500)

Tuberous sclerosis complex (TSC) is a tumor suppressor gene syndrome characterized by seizures, mental retardation, autism, and tumors in the brain, retina, kidney, heart, and skin. Angiomyolipomas are benign tumors with three distinct components: smooth muscle cells, adipose cells, and abnormal blood vessels.¹ Mutations in two genes, *TSC1* on chromosome 9q34² and *TSC2* chromosome 16p13,³ cause TSC. Loss of heterozygosity (LOH) in the *TSC1* or *TSC2* region occurs in most angiomyolipomas, rhabdomyomas, and astrocytomas from TSC pa-

tients.⁴ *TSC2* LOH also occurs in 10% of sporadic angiomyolipomas⁵ and in 60% of angiomyolipomas from women with the sporadic form of lymphangiomyomatosis (LAM).⁶ Although it is known that the smooth muscle and fat components of angiomyolipomas have *TSC1* or *TSC2* LOH, whether the dysplastic vessels within the angiomyolipomas have LOH is an area of uncertainty. We have previously found that angiomyolipoma vessels from two TSC patients did not contain the second hit somatic genetic event, indicating that they are not neoplastic.⁷ Recently, however, another group found the opposite result in one angiomyolipoma from a TSC patient.⁸ Here, we studied angiomyolipomas from patients with the sporadic form of LAM. In these angiomyolipomas, we identified five distinct morphological types of vessels. Four of the vessel types had *TSC2* LOH, and are neoplastic. One vessel type lacked *TSC2* LOH, and is therefore non-neoplastic. The endothelial cells also lacked LOH. All of the neoplastic components of the tumor, as defined by the LOH analysis, showed hyperphosphorylation of the ribosomal protein S6 relative to the non-neoplastic components of the tumor. Angiomyolipomas may be the first example of a human tumor in which formation of blood vessel walls by tumor cells has been demonstrated.

Materials and Methods

Patients

This study was approved by the Institutional Review Board of Fox Chase Cancer Center. All four patients (patients 436, 437, 487, and 492) have the sporadic form of lymphangiomyomatosis and each had a single renal angiomyolipoma. The patients ranged in age from 20 to 39 years at the time of angiomyolipoma resection. The angiomyolipomas had maximum dimensions ranging from 9.5 to 22 cm. Loss of heterozygosity in these angiomyolipomas has been previously reported.⁶

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Figure 1. Angiomyolipomas contain five morphological vessel types. Cellular vessels (**A**, magnification, $\times 400$) and collagenous vessels (**B**, magnification, $\times 400$) have thick walls and similar lumen sizes, but the wall of cellular vessels is highly cellular while the wall of collagenous vessels is hypocellular. Hemangiopericytic vessels (**C**, magnification, $\times 400$) have a thin wall and branching or irregularly shaped lumen. Glomeruloid vessels (**D**, magnification, $\times 400$) have cellular walls with a clustered appearance and small lumens. Aneurysmatic vessels (**E**, magnification, $\times 400$) have irregular hypocellular walls, often with aneurysm-like dilatations. In each panel, the wall thickness of the vessel is indicated.

Immunohistochemistry

Paraffin sections were deparaffinized and rehydrated. For antigen retrieval, sections were boiled in Citric Buffer (10 mmol/L sodium citrate- trisodium salt dihydrate, Sigma, St. Louis, MO), pH 6.0, at 750 W for 10 minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 30 minutes at room temperature.

Non-specific background was eliminated by incubating the tissue with normal goat serum (Super Sensitive Kit, BioGenex, San Ramon, CA) for 30 minutes at room temperature. The sections were then incubated in a humidified chamber with mouse monoclonal antibodies against desmin, vimentin, muscle-specific actin (α and γ iso-types, all from BioGenex), or rabbit polyclonal antibodies

Figure 2. Histochemical and immunohistochemical differences between cellular and collagenous vessels. Collagenous vessels frequently contained scattered, irregularly positioned larger cells, indicated with an **arrow** (**A**, magnification, $\times 400$, **B**, magnification, $\times 1000$). Masson trichrome staining demonstrates that collagenous vessels have abundant collagen deposition (**C**, magnification, $\times 200$) while cellular vessels have little extracellular collagen (**D**, magnification, $\times 100$). Desmin immunoreactivity was absent in the walls of collagenous vessels (**E**, magnification, $\times 400$) but strongly positive in cellular vessels (**F**, magnification, $\times 400$).

against phospho-S6 ribosomal protein (Cell Signaling Technology, Beverly, MA), then rinsed and incubated with biotinylated goat anti-mouse antibody (BioGenex) for 30 minutes at room temperature. Visualization was performed using streptavidin-peroxidase (BioGenex). Sections were counterstained with Gill's hematoxylin.

Histochemistry

Slides were prepared with Masson trichrome staining for evaluation of collagen deposition and with periodic acid-Schiff (PAS) stain (with and without diastase) for evaluation of glycogen deposition, using standard methods.

Table 1. Histochemical and Immunohistochemical Comparison of Angiomyolipoma Cell and Vessel Types

Cell type or vessel type	Collagen deposition	Glycogen deposition	Vimentin	Muscle-specific actin	Desmin	HMB-45	Phospho-S6 ribosomal protein
Angiomyolipoma smooth muscle cells	+ focally between stromal cells	+	+	+	+	+	+
Adipose tissue	—	+ focally	+	+	+	+	+
Cellular vessels	—	+	+	+	+	+	+
Hemangiopericytic vessels	—	+	+	+	+	+	+
Glomeruloid vessels	—	+	+	+	—	+	+
Aneurysmatic vessels	+	+	+	+	—	+ focally	+
Collagenous vessels	+	+	+	+	—	—	—
Normal renal blood vessels	—	—	+	+	+	—	—

Laser Capture Microdissection and DNA Extraction

Different types of vessels were identified morphologically on hematoxylin and eosin (H&E) stained slides. Laser capture microdissection (PixCell II, Arcturus Engineering, Mountain View, CA) was used to isolate cells from the smooth muscle and fat components of the angiomyolipoma, and cells from the walls of each vessel type. The endothelial cell layer was avoided (Figure 1,A and B). For the angiomyolipoma from patient 437, we were able to separately capture endothelial cells from collagenous, cellular, and aneurysmatic vessels. For all specimens, DNA was extracted by overnight incubation in 30 μ l of extraction buffer (0.5% Tween 20, 0.2 mg/ml Proteinase K, 0.05 mol/L Tris-HCl (pH 8.9), 2 mmol/L EDTA, and 1.0 mmol/L NaCl).

Loss of Heterozygosity Analyses

A panel of microsatellite markers near the *TSC2* locus on chromosome 16p13 was used: D16S287, D16S291, D16S418, D16S749, and Kg8 (Research Genetics, Huntsville, AL). LOH analyses were performed using a 2.5 μ l aliquot of the DNA solution in a 10- μ l polymerase chain reaction.⁴ The PCR amplification consisted of 95°C for 5 minutes followed by 40 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 45 seconds, and a final extension at 72°C for 10 minutes. PCR was performed with radioactive phosphorus-labeled deoxyguanosine triphosphate in the reaction mix. TaqStart antibody (Clontech, Palo Alto, CA) at final concentration of 0.056 μ mol/L was used to enhance specificity. The PCR products were resolved by denaturing 8 mol/L urea polyacrylamide gel electrophoresis (Gibco, Grand Island, NY) and were visualized by autoradiography. All results were repeated at least twice for confirmation.

Results

Angiomyolipomas Contain Multiple Vessel Types

In H&E stained sections, we identified five morphological types of vessels in the angiomyolipomas. Two of the vessel types, which we will refer to as cellular vessels and collagenous vessels, have thick walls and large lumens. Cellular vessels have thick, highly cellular walls (Figure 1A). Collagenous vessels have hypocellular walls with abundant extracellular material (Figure 1B). Three of the four angiomyolipomas in this study contained cellular vessels and three of the four contained collagenous vessels. The cellular and collagenous vessels were large, with a typical wall thickness of 200 to 400 μ m and lumen size of 500 to 800 μ m. The walls of cellular vessels usually contained cells with clear cytoplasm and a spindle or polygonal shape, similar in appearance to the angiomyolipoma's smooth muscle component. In contrast, cells in the walls of collagenous vessels tended to be more elongated, with dense pink cytoplasm.

Collagenous angiomyolipoma vessels have been previously described,^{1,9} but we did not find previous reports of cellular vessels. In some collagenous vessels we observed the apparent infiltration into the vessel wall of larger cells with the appearance of the angiomyolipoma's smooth muscle cell component (Figure 2,A and B). These cells were present to varying degrees in about 30% of collagenous vessels. The relationship of these larger cells to the tendency of benign angiomyolipomas to spread to regional lymph nodes¹ and the possible spread of angiomyolipoma cells to the lung in patients with sporadic LAM¹⁰ is not known.

Three types of vessels with thinner walls were identified. The first, hemangiopericytic vessels (Figure 1C), were often branching, with typical wall thickness around 50 μ m. These vessels were identified in three of the angiomyolipomas. Glomeruloid vessels (Figure 1D) are often present within the fat component of angiomyolipoma. Glomeruloid vessels have the smallest lumen of

Figure 3. Immunohistochemical features of the different vessel types. Vimentin and smooth muscle actin reactivity was present in cellular (**A** and **B**, magnification, $\times 100$), collagenous (**D** and **E**, magnification, $\times 100$), hemangiopericytic (**G** and **H**, magnification, $\times 200$), glomeruloid (**J** and **K**, magnification, $\times 200$) and aneurysmatic vessels (**M** and **N**, magnification, $\times 100$). Desmin reactivity was present only in cellular (**C**, magnification, $\times 100$) and hemangiopericytic vessels (**I**, magnification, $\times 200$). Negative desmin reactivity was observed in collagenous (**F**, magnification, $\times 100$), glomeruloid (**L**, magnification, $\times 200$) and aneurysmatic vessels (**O**, magnification, $\times 100$).

any of the vessel types (typically 50 to 80 μm) with a cellular wall with thickness of 40 to 50 μm . The third type of thin-walled vessel, aneurysmatic vessels (Figure 1E) have fibrous, hypocellular walls, which sometimes create small dilatations or aneurysms, with typical wall thickness between 60 to 80 μm . Hemangiopericytic and glomeruloid vessels have been previously described only in atypical epithelioid angiomyolipomas from patients without TSC.¹¹ Aneurysmatic and glomeruloid vessels were each identified in two of the angiomyolipomas.

Masson trichrome staining revealed abundant collagen deposition in the wall of the collagenous (Figure 2C) and aneurysmatic vessels (data not shown), but not cellular vessels (Figure 2D), glomeruloid vessels, or hemangiopericytic vessels. PAS staining revealed fine, diastase-negative glycogen granules deposited in the angiomyolipoma's smooth muscle component and in the cells lining all five types of vessels (data not shown). The angiomyolipoma's smooth muscle cell and fat components and the cells within all five vessel types were immunoreactive with muscle-specific actin and with vimentin (data not shown). Desmin immunoreactivity was present in the smooth muscle and adipose components of the angiomyolipomas, as well as in the cells lining the cellular vessels (Figure 2F) and the hemangiopericytic vessels, but not the collagenous (Figure 2E), aneurysmatic, or glomeruloid vessels. Smooth muscle cells from normal renal blood vessels were immunoreactive for vimentin, desmin, and muscle-specific actin. The immunohistochemical features of the different vessel types are summarized in Table 1 and illustrated in Figure 3.

Loss of Heterozygosity Analyses

Each of the angiomyolipomas in this study was previously found to have LOH in the *TSC2* region of chromosome 16p13.⁶ Laser capture microdissected specimens of smooth muscle and adipose components were analyzed separately for LOH using a panel of microsatellite markers near the *TSC2* gene. In each case, the angiomyolipoma smooth muscle cell and adipose components had LOH for multiple markers. We next analyzed microdissected cells from the wall of each vessel type for LOH (Figure 4, Table 2). The angiomyolipoma from patient 436 contained cellular vessels, hemangiopericytic vessels, and collagenous vessels. The cellular and hemangiopericytic vessels had LOH at four markers, and the collagenous vessels did not have LOH. The angiomyolipoma from patient 437 contained cellular vessels, hemangiopericytic vessels, glomeruloid vessels, and collagenous vessels. The cellular, hemangiopericytic, and glomeruloid vessels had LOH at two markers, while the collagenous vessels did not have LOH. The angiomyolipoma from patient 487 contained cellular vessels and aneurysmatic vessels, both of which had LOH at three markers. The angiomyolipoma from patient 492 contained aneurysmatic vessels, hemangiopericytic vessels, glomeruloid vessels, and collagenous vessels. The aneurysmatic, hemangiopericytic, and glomeruloid vessels

Figure 4. LOH is present in four of the five angiomyolipoma vessel types. Examples of chromosome 16p13 LOH analyses are shown for each of the different vessel types. Beneath each panel, the patient number and marker are provided. The location of the primary band representing each of the two alleles is indicated with a line in the normal kidney (NK). The "lost" allele is indicated with an **arrow** in non-microdissected angiomyolipoma (A). LOH was seen in the microdissected angiomyolipoma smooth muscle cell component (SM) and fat component (F), and in cellular vessels (CeV), hemangiopericytic vessels (HV), aneurysmatic vessels (AV), and glomeruloid vessels (GV). LOH was not detected in normal kidney (NK) or in collagenous vessels (CoV).

had LOH at two markers, and the collagenous vessels did not have LOH.

We next analyzed microdissected endothelial cells from three vessel types. Because of the need to collect a sufficient number of cells (at least 250 nuclei) for reliable LOH analyses, we were limited to angiomyolipomas with enough vessels of a single type to allow pooling of endothelial cells from different vessels. Therefore, the endothelial cell analysis was performed only on cellular, aneurysmatic, and collagenous vessels from patient 437. LOH was not present in the endothelial cells (Figure 5).

In summary, for each of the angiomyolipomas, LOH was present in the smooth muscle cells lining cellular, hemangiopericytic, aneurysmatic, and glomeruloid vessels (Table 2), indicating that they are part of the neoplastic component of the tumor. In contrast, endothelial cells from cellular and aneurysmatic vessels did not have LOH, suggesting that the endothelial component of these vessels arises independently. LOH was not present in

Table 2. Loss of Heterozygosity Results for Individual Chromosome 16p13 Markers

Patient	Tissue	D16S749	D16S287	D16S418	D16S291	Kg8
436	Angiomyolipoma*	— [†]	LOH [‡]	LOH	LOH	LOH
	Smooth muscle cells	—	LOH	LOH	LOH	LOH
	Adipose tissue	—	LOH	LOH	LOH	LOH
	Cellular vessels	—	LOH	LOH	LOH	LOH
	Hemangiopericytic vessels	—	LOH	LOH	LOH	LOH
	Glomeruloid vessels	—	—	—	—	—
	Aneurysmatic vessels	—	—	—	—	—
	Collagenous vessels	—	2 [§]	2	2	2
437	Angiomyolipoma*	2	2	2	LOH	LOH
	Smooth muscle cells	2	2	2	LOH	LOH
	Adipose tissue	—	—	—	—	—
	Cellular vessels	2	2	2	LOH	LOH
	Hemangiopericytic vessels	2	2	2	LOH	LOH
	Glomeruloid vessels	2	2	2	LOH	LOH
	Aneurysmatic vessels	—	—	—	—	—
	Collagenous vessels	2	2	2	2	2
487	Endothelial cells	—	—	—	2	2
	Angiomyolipoma*	LOH	1 [¶]	LOH	1	LOH
	Smooth muscle cells	LOH	1	LOH	1	LOH
	Adipose tissue	—	—	—	—	—
	Cellular vessels	LOH	1	LOH	1	LOH
	Hemangiopericytic vessels	—	—	—	—	—
	Glomeruloid vessels	—	—	—	—	—
	Aneurysmatic vessels	LOH	1	LOH	1	LOH
492	Collagenous vessels	—	—	—	—	—
	Angiomyolipoma*	—	2	LOH	LOH	1
	Smooth muscle cells	—	—	—	—	—
	Adipose tissue	—	2	LOH	LOH	1
	Cellular vessels	—	—	—	—	—
	Hemangiopericytic vessels	—	2	LOH	LOH	1
	Glomeruloid vessels	—	2	LOH	LOH	1
	Aneurysmatic vessels	—	2	LOH	LOH	1
	Collagenous vessels	—	2	2	2	1

*Non-microdissected tissue; [†]vessels of this type not present in this case, or this marker not typed for this patients; [‡]LOH: loss of heterozygosity; [§]2: retention of heterozygosity; [¶]1: non-informative marker. The markers are listed in centromeric to telomeric order.

collagenous vessels, or in the endothelial cells lining collagenous vessels.

Ribosomal Protein S6 (S6) is Hyperphosphorylated in the Neoplastic Component of Angiomyolipomas

Recently, S6 hyperphosphorylation was found in cells lacking tuberlin expression¹² and in cells lacking hamartin expression,¹³ indicating that the tuberlin-hamartin complex negatively regulates the activity of S6 Kinase. We immunostained the angiomyolipomas with an antibody specific for phospho-S6. The cell types in which LOH was detected (the angiomyolipoma fat, smooth muscle cells and the cells lining the cellular, hemangiopericytic, glomeruloid, and aneurysmatic vessels) (Figure 6,A and B, D–F) were immunoreactive with anti-phospho S6 antibody. The endothelial cells lining these vessels, which did not have LOH, were not immunoreactive. The collagenous vessels, which did not have LOH, were also not immunoreactive (Figure 6C).

Discussion

TSC is a tumor suppressor gene disorder in which two of the most frequent tumors, renal angiomyolipomas and

facial angiofibromas, have a prominent vascular component. Whether these vessels are reactive (resulting from the proliferation of normal cells in response to tumor-secreted factors) or neoplastic (consisting of tumor cells) is controversial, with prior studies providing conflicting data.^{7,8} We examined the genetic basis of angiomyolipoma vessels in patients with the sporadic form of pulmonary lymphangiomyomatosis (LAM). Sporadic LAM angiomyolipomas frequently have somatic inactivation of

Figure 5. Angiomyolipoma endothelial cells do not have LOH. The location of the primary band representing each of the two alleles is indicated with a line in the normal kidney (NK). The “lost” allele is indicated with an **arrow** in non-microdissected angiomyolipoma (AML). LOH was not detected in microdissected endothelial cells from cellular vessels (CeV), aneurysmatic vessels (AV), or collagenous vessels (CoV).

Figure 6. Hyperphosphorylation of S6 ribosomal protein is present in the neoplastic components of the angiomyolipomas. Phospho-S6 ribosomal protein immunoreactivity was present in the angiomyolipoma fat component (**A**, magnification, $\times 400$, **arrowheads**), in the smooth muscle component (**B**, asterisk, magnification, $\times 400$), and in cells in the walls of cellular vessels (**B**, magnification, $\times 400$), hemangiopericytic vessels (**D**, magnification, $\times 400$), glomeruloid vessels (**E**, magnification, $\times 400$), and aneurysmatic vessels (**F**, magnification, $\times 400$), but not in the walls of collagenous vessels (**C**, magnification, $\times 400$). Endothelial cells (**arrows** in **panels B, C, D, E, and F**) were not immunoreactive. In each **panel**, the wall thickness of the vessel is indicated.

both alleles of the *TSC2* gene.^{6,10} We were surprised to find that the angiomyolipomas contained five vessel types, one of which (cellular) has not to our knowledge been previously recognized and two of which (glomeru-

loid and hemangiopericytic) have been previously recognized only within sporadic atypical epithelioid angiomyolipomas.¹¹ The angiomyolipoma vessels had clear morphological differences in diameter, wall thickness,

lumen size, aneurysmic wall dilatations, collagen deposition, and glycogen deposition. These differences may indicate that the TSC proteins play a functional role in the normal development and maturation of blood vessels.

We separately microdissected cells from the walls of each vessel type, as well as cells in the smooth muscle and fat components of the angiomyolipomas, and analyzed the DNA for LOH in the *TSC2* region of chromosome 16p13. The smooth muscle and fat components of the angiomyolipomas had LOH, as expected.^{7,8} Four of the five vessel types (cellular, hemangiopericytic, glomeruloid, and aneurysmatic) also had LOH, and in each case the LOH pattern was identical to the LOH pattern in the smooth muscle and fat cells. This indicates that the cells in the walls of these vessels are neoplastic, and supports a model in which angiomyolipomas are derived from a mesenchymal cell that retains the ability to differentiate into multiple different lineages.¹⁴ In contrast, collagenous vessels did not have LOH, and LOH was not detected in endothelial cells from cellular, aneurysmatic vessels, or collagenous vessels. The lack of LOH in the endothelial cells may be related to the previously demonstrated expression of vascular endothelial growth factor (VEGF) by angiomyolipomas,¹⁵ which could be involved in the recruitment of endothelial cells. Taken together, these results demonstrate that multiple genetic mechanisms contribute to angiomyolipoma blood vessel formation, including both neoplastic and non-neoplastic vessel wall formation and the recruitment of non-neoplastic endothelial cells.

To our knowledge, this is the first time that the vascular component of a benign vascular tumor has been shown to be neoplastic.¹⁶ Studies of von Hippel Lindau-associated hemangioblastomas and retinal angiomas have consistently demonstrated that the stromal cell component, and not the vascular component, contains the second hit mutation.^{17–19} TSC, therefore, appears to reflect a novel genetic mechanism of blood vessel formation.

Tuberin, the *TSC2* gene product,²⁰ and hamartin, the *TSC1* gene product,² have been shown to interact^{21,22} and appear to be involved in multiple cellular pathways. Hamartin interacts with the ezrin-radixin-moesin (ERM) family of cytoskeletal proteins and activates the GTPase Rho.²³ Since Rho is known to regulate vascular smooth muscle cell contraction, differentiation, and proliferation,^{24–26} it is interesting to speculate that aberrant signaling involving Rho contributes to blood vessel formation in angiomyolipomas.

Recently, hyperphosphorylation of p70 S6 Kinase (S6K) and its substrate ribosomal protein S6 was observed in cells lacking hamartin from a murine model of *Tsc1*,¹³ and in cells lacking tuberin from the Eker rat model of *Tsc2*,¹² suggesting that the tuberin-hamartin complex negatively regulates S6K. S6K is a critical component of the tightly regulated signal transduction pathways controlling cell size and integrating the external availability of nutrients with protein synthesis (reviewed in^{27–29}). We found that all of the components of the angiomyolipomas in which LOH was detected were immunoreactive with an antibody to phospho-S6. In contrast, the components in which LOH was not detected, includ-

ing endothelial cells, were not immunoreactive. This is consistent with a model in which hyperphosphorylation of S6 is involved in the pathogenesis of vascular structures within angiomyolipomas. Signaling through S6K is believed to be involved in the proliferation and migration of human vascular smooth muscle cells, and the structural remodeling of vessel walls.^{30–32}

In summary, we found that angiomyolipomas contain multiple vessel types, four of which (cellular, hemangiopericytic, glomeruloid, and aneurysmatic) have LOH. One vessel type (collagenous) does not have LOH. The presence of LOH in all three components of angiomyolipomas (vessels, fat, and smooth muscle) supports the hypothesis that angiomyolipomas arise from a mesenchymal precursor cell that retains differentiation plasticity. This distinguishes angiomyolipomas from other benign blood vessel-filled tumors such as those in von Hippel Lindau disease, in which the stromal cells are neoplastic and the vascular cells are not. The presence of hyperphosphorylated ribosomal protein S6 within the neoplastic components of the angiomyolipomas suggests that signaling pathways involving S6K contribute to the formation of the vascular structures within angiomyolipomas.

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