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Activation of the mTOR Pathway in Sporadic Angiomyolipomas and Other Perivascular Epithelioid Cell Neoplasms*

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Abstract

Angiomyolipomata (AML) belong to a family of tumors known as perivascular epithelioid cell tumors (PEComas) that share a common immunophenotypic profile of muscle and melanocytic differentiation. These tumors are clonal in nature and have a strong association with tuberous sclerosis. Genetic analyses have reported allelic imbalance at the *TSC2* locus on 16p13. In the context of non-TSC, non-LAM associated AMLs and non-renal PEComas, the functional status of the TSC2 signaling pathway has not been reported. Studies over the last several years have uncovered a critical role of the *TSC1/2* genes in negatively regulating the Rheb/mTOR/p70S6K cascade. Here, we examined the activity of this pathway in sporadic AMLs and PEComas using immunohistochemical and biochemical analyses. We found increased levels of phospho-p70S6K, a marker of mTOR activity, in 15 of 15 non-TSC AMLs. This was accompanied by reduced phospho-AKT expression, a pattern that is consistent with the disruption of TSC1/2 function. Western blot analysis confirmed mTOR activation concurrent with the loss of TSC2 and not TSC1 in sporadic AMLs. Similarly, elevated phospho-p70S6K and reduced phospho-AKT expression was detected in 14/15 cases of extra-renal PEComas. These observations provide the first functional evidence that mTOR activation is common to sporadic, non-TSC-related AMLs and PEComas. This suggests the possibility that mTOR inhibitors such as rapamycin may be therapeutic for this class of disease.

Keywords

AML; PEComa; immunohistochemistry; tuberous sclerosis; TSC2; p70S6K

Introduction

The World Health Organization recently recognized as a distinct entity a heterogeneous family of neoplasms with perivascular epithelioid cell differentiation known as PEComa¹. These lesions are defined as “mesenchymal tumors composed of histologically and immunohistochemically distinctive perivascular epithelioid cells”. While the origin of these cells is unknown, PEComas include angiomyolipoma (AML), lymphangio-leiomyomatosis

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(LAM), clear cell ‘sugar’ tumor of the lung, clear cell myomelanocytic tumor of the falciform ligament, and other “unusual clear cell tumors” designated as PEComas not otherwise specified (NOS)². Such tumors may be conceptualized as monotypic epithelioid or spindled non-renal AML, akin to the monotypic tumors known to occur in TSC-associated renal AML. The clinical course of these tumors is highly variable ranging from indolent benign lesions, to lesions with an aggressive clinical course including distant metastases. Due to their relative scarcity, little is known about the underlying molecular mechanism. Currently, there is no effective medical therapy.

As suggested by its designation, perivascular epithelioid cells (PEC) are characterized histologically by their epithelioid appearance and their physical relationship to blood vessels¹. They display a distinct immunophenotype that includes the expression of melanocytic and smooth muscle markers (e.g., gp100 and smooth muscle actin) but not epithelial antigens. Since the PECs have no normal anatomic counterpart, the origin of these tumors remains elusive.

AML and LAM represent a subset of PEComas that are strongly associated with tuberous sclerosis complex (TSC)³. The latter is an autosomal dominant disease characterized by the multi-system development of benign tumors stemming from underlying mutations of either the TSC1 or TSC2 tumor suppressor gene⁴. AML of the kidney develops in >50% of TSC patients and is a major source of morbidity secondary to hemorrhage and destruction of renal parenchyma. Recent studies suggest that up to one-third of adult TSC females also have manifestations of LAM based on radiologic characteristics of their lungs^{5, 6}. LAM occurs almost exclusively in females, although one case of LAM in a male patient has been reported⁷. The coexistence of AML and LAM in TSC suggests a common pathogenetic mechanism stemming from the disruption of the TSC1/2 pathway. However, it is not known if sporadic AMLs and other less common forms of PEComas suffer from the functional consequences of the loss of TSC1/2 activity.

A small number of studies that have examined the cytogenetic and molecular genetic features of PEComas have highlighted the clonal nature of these tumors but without consistent findings. In the context of the TSC genes, allelic loss of the TSC2 locus on 16p13 has been found repeatedly in sporadic AMLs and PEComas suggesting a potential causal link^{8–11}. However, many other recurrent chromosomal alterations including the loss of 1p, 17p, 18p, 19 as well as gain of 2q, 3q, 5, 12q and X have been identified in a recent comparative genomic hybridization study of PEComas¹². To better appreciate the functional significance of 16p LOH in these tumors, we used a biochemical and immunohistochemical (IHC) approach to assess the potential role of the TSC2 pathway.

Proteins encoded by the *TSC1* and *TSC2* genes, hamartin and tuberlin respectively, function as a complex to negatively regulate mTOR signaling in response to multiple environmental signals¹³. In the presence of growth factors, PI3K-mediated AKT activity promotes Rheb-dependent mTOR function to increase protein synthesis and consequently, cell growth (Figure 1A). This pathway is under the regulation of three tumor suppressor proteins, PTEN, TSC1, and TSC2. PTEN functions as a phosphatase to reduce intracellular lipid products (e.g., PIP₃) of PI3K, and in doing so, blocks the activation of AKT¹⁴. The TSC1/2 complex acts downstream of AKT to promote hydrolysis of Rheb-GTP via its GAP activity, which leads to the suppression of mTOR signaling. The mTOR serine/threonine kinase phosphorylates p70S6K and PHAS1 to promote ribosomal biogenesis and protein translation¹⁵. Recent studies identified other tumor suppressor proteins, LKB1 (Puetz-Jeghers syndrome) and NF1 (neurofibromatosis 1) that also regulate the mTOR pathway by modulating the activity of TSC1/2^{16,17}. Thus, dysregulation of the TSC/mTOR pathway may be central to a diverse group of human tumors.

While the loss of PTEN, TSC1 or TSC2 leads to mTOR activation during tumor development, there is a unique difference that distinguishes the molecular events in the respective tumors. When PTEN is absent, AKT becomes constitutively activated (i.e., high phospho-AKT levels), whereas AKT phosphorylation is suppressed when either TSC1 or TSC2 is lost or mutated (Figures 1B,C). The opposing effects on AKT activity in these two scenarios have been implicated in the observed differences in phenotypic behavior (i.e., the benign nature of TSC-related tumors versus the frequent involvement of PTEN in human cancers)^{18,19}. The mechanism of AKT inhibition following disruption of TSC1/2 has been shown to be secondary to the negative feedback of p70S6K on the insulin receptor substrate 1, IRS1, leading to reduced insulin sensitivity^{20, 21}. Therefore, the combined assessment of mTOR and AKT activities in tumors provides not only evidence for the possible involvement of the mTOR pathway but also clues to the underlying mechanisms. In this study, we show that sporadic forms of renal AML and non-renal PEComas possess high levels of phospho-p70S6K and low levels of phospho-AKT consistent with the inactivation of TSC1/2. Indeed, our data indicate the loss of TSC2 but not PTEN expression in sporadic AMLs.

Materials and Methods

Tissue samples

Kidney AML samples (formalin fixed, paraffin embedded) were obtained from Elizabeth Henske MD, Fox Chase Cancer Center (Philadelphia, PA) and from the University of Washington Medical Center (Seattle, WA). PEComa samples (formalin fixed, paraffin embedded) were obtained from Emory University (Atlanta, GA) and the University of Washington Medical Center (Seattle, WA). Fresh AML and kidney tissues were obtained from the University of Washington Medical Center, Seattle, WA. Samples were obtained through protocols approved by the respective Institutional Review Boards.

Chemicals and Reagents

The following antibodies were purchased from the respective suppliers: rabbit anti-PHAS-I (4E-BP1) from Zymed (San Francisco, CA), rabbit anti-tuberin (C-20) from Santa Cruz Biotechnology Inc. (Santa Cruz, CA), rabbit anti-LKB1 from Calbiochem (La Jolla, CA), and mouse anti-actin from Sigma Chemical Co. (St. Louis, MO). All other antibodies were purchased from Cell Signaling (Beverly, MA). Secondary antibodies and ECL reagent were purchased from Amersham Pharmacia Biotech (Piscataway, NJ). The Elite ABC kits, DAB, and Hematoxylin QS were purchased from Vector Laboratories (Burlingame, CA). Lambda Protein Phosphatase (λ -Ppase) was purchased from NE Biolabs (Ipswich, MA).

Immunohistochemistry

Five-micrometer tissue sections on glass slides were deparaffinized, rehydrated, and washed with PBS. Following antigen retrieval in 0.1 mM sodium citrate (pH 6.0) and quenching of endogenous peroxidase activity with 3% H₂O₂, samples were blocked with 5% normal goat serum (NGS) or 5% normal horse serum (NHS) prior to incubation with primary antibodies overnight at 4 °C. Negative controls were treated with 5% NGS or NHS without primary antibodies. Signals were processed according to supplied protocol (Elite ABC Kit). Slides were counterstained with Hematoxylin QS, dehydrated and mounted using Permount (Fischer Scientific, Santa Clara, CA).

Results of the immunohistochemistry were interpreted using the following scale: 0, no staining; 1, focal (<50%), weak staining; 2, diffuse (>50%), weak staining; 3, focal, intense staining; 4, diffuse, intense staining. Staining intensity was graded with respect to the adjacent non-tumor elements.

Western Blotting

Both AML and corresponding normal kidney tissue were homogenized in ice-cold radioimmunoprecipitation (RIPA) buffer (1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 10 mM Tris (pH 7.2), 0.025 M β -glycophosphate (pH 7.2), 2mM EDTA, and 50mM sodium fluoride) with protease and kinase inhibitors (0.05 mM AEBSF, 10 μ g/ml aprotinin, 10 μ g/ml pepstatin, 1 mM orthovanadate, 10 μ g/ml leupeptin, 1 mM microcystin LR). The protein concentration was measured using the BCA Protein Assay (Pierce, Rockford, IL). Equal amounts of protein were separated by SDS-PAGE, transferred to Immobilon-P membranes (Millipore, Bedford, MA) and blotted with antibodies according to manufacturer recommendations. Densitometry of bands was performed using Image J. For phosphatase treatment experiments, tissue lysates from human AML and corresponding normal kidney containing 80 μ g of protein were treated with 400 units of λ -PPase and incubated at 30°C for 20 minutes prior to SDS-PAGE.

Results

mTOR signaling is up-regulated in sporadic AMLs

The levels of p70S6K and AKT phosphorylation are commonly used to indicate their activities. In this study, we used phospho-specific antibodies in immunohistochemical (IHC) analyses to dissect the mTOR pathway. Previously, we have shown that TSC-related AMLs exhibit evidence of mTOR activation^{22, 23}. As an example, Figures 2A and 2B illustrate the use of phospho-specific antibodies in a case of renal AML from a TSC patient with known TSC2 mutation. With the loss of TSC2 function, mTOR signaling in the tumor was increased as illustrated by the strong immunoreactivity to anti-phospho-p70S6K (Thr389) antibody (Figure 2A). Consequently, this led to a feedback inhibition of AKT (see Figure 1) resulting in the lack of phospho-AKT (Ser473) expression (Figure 2B). In contrast, a sample of human prostate cancer, which frequently incurs PTEN loss, stained strongly for phospho-AKT (Ser 473) indicating increased AKT activity. Thus, the immunophenotype based on reactivity to phospho-p70S6K and phospho-AKT antibodies can provide an assessment of mTOR activity as well as clues to the underlying mechanism.

Using this approach, we examined the status of p70S6K and AKT in a set of 15 sporadic renal AMLs. The formalin-fixed, paraffin-embedded tissues were obtained from patients with histologically confirmed AMLs with no personal or family history of TSC or LAM. In accordance with previous reports, the majority of the AMLs were derived from women (12 of 15), ages ranging between 29 and 80. The staining intensity and distribution within the AMLs were compared to adjacent normal kidneys and scored from 0 to 4+ (see Materials and Methods). Diffuse strong immunoreactivity (4+) to anti-phospho-p70S6K antibody was seen in all 15 samples of sporadic AMLs examined. Within each tumor, all three components (epithelioid cells, adipocyte-like cells and cells of abnormal blood vessels) expressed phospho-p70S6K. Figure 3A illustrates two examples of phospho-p70S6K staining specifically in the tumors but not the adjacent kidneys. As previously noted, a population of cells within the normal collecting ducts of the kidney expressed phospho-p70S6K²². To confirm that p70S6K activity in these lesions is elevated, we analyzed the expression of the phosphorylated form of its substrate, ribosomal S6 protein, in a random subset of these AMLs. In the 5 cases examined, all displayed significant phospho-S6 staining (3 or 4+) consistent with increased p70S6K activity (Figure 3B). In terms of AKT, none of the 15 sporadic AMLs showed significant immunoreactivity to anti-phospho-AKT(Ser473) antibody (0+) (Figure 3C), while staining for total AKT remained unchanged compared to adjacent normal kidneys (data not shown). These findings demonstrate a highly consistent immunophenotype of sporadic AMLs showing increased mTOR and decreased AKT activities that are consistent with the loss of TSC1/2 function.

Loss of TSC1/2 in sporadic AMLs

To further define the mTOR signaling in AMLs, we examined other components of the pathway as outlined in Figure 1 in four independent AML cases by Western blot analyses. Fresh tissues were obtained from nephrectomy specimens. The indications for surgery were symptom-related in three (i.e., pain, hemorrhage) and to rule out malignancy in one. None of the four patients has personal or family history of TSC or LAM. Ages ranged from 32 to 80; all were females. The tumor sizes were 1.2 cm, 7.5 cm, 13 cm and 15 cm.

First, to confirm the findings of our IHC analysis, we analyzed the expression of AKT and down-stream effectors of mTOR: S6K, S6 and PHASI. As shown in Figure 4A, each of the sporadic AMLs expressed higher levels of phospho-p70S6K(Thr389) and phospho-S6 (Ser235/236) compared to adjacent normal kidneys. Further, AMLs expressed slower mobility forms of PHASI indicative of its hyper-phosphorylation state compared to normal kidney. The combined presence of phosphorylated S6, p70S6K and PHASI strongly implicates elevated mTOR activity in these tumors.

To determine the state of AKT function, phosphorylation of Ser473 and Thr308, two sites that are necessary for AKT activity, were examined. Figure 4A shows that all but one AML tested have significantly reduced levels of phospho-AKT expression despite comparable amounts of total AKT between AML and normal kidney. Case 2 displayed 67% of the normal kidney level when normalized to total AKT, also indicating reduced AKT activity in the AML compared to normal kidney. Thus, the Western blotting results are in keeping with the IHC analyses confirming the up-regulation of mTOR and down-regulation of AKT in these lesions. The inverse relationship between AKT and mTOR is highly suggestive of a functional loss of TSC1/2.

To explore the mechanism of mTOR activation in these tumors, we evaluated the status of four pathways that have an influence on mTOR signaling including TSC1/2, PTEN, LKB1/AMPK and NF1/Ras/ERK (see Figure 1). With respect to TSC1/2, Western blotting analysis showed a dramatic reduction in TSC2 expression relative to the adjacent kidney tissues suggestive of the loss of TSC2 (Figure 4B). The faint TSC2 band found in the AML samples represents the non-tumor elements of the AMLs (e.g., connective tissues, non-tumor vessels). On the contrary, TSC1 was expressed in all four AMLs, but the immunoreactive bands representing TSC1 migrated slower on SDS-PAGE analyses, thus giving the appearance of broader and less distinct bands (Figure 4B). This is a reflection of the phosphorylation-dependent mobility shifts of TSC1 when it is unbound to TSC2. In support of this, when we treated the tumor lysates with λ phosphatase, the tumor TSC1 bands reverted to the form found in the non-tumor tissues (data not shown). We observed the same phenomenon in *Tsc2*^{-/-} embryonic fibroblasts or when TSC1 alone was over-expressed in Cos7 cells (data not shown). Together, these observations showed the consistent loss of TSC2 but not TSC1 expression in sporadic AMLs.

To assess other plausible causes for mTOR activation in AMLs, we examined other components of the pathway outlined in Figure 1. Loss of PTEN would result in activation of mTOR, but we found PTEN to be expressed at significantly higher levels in all 4 AMLs compared to normal kidneys (Figure 4B), therefore unlikely to contribute to mTOR deregulation. LKB1 expression was more variable in these lesions with a slight, overall reduction in its levels. To evaluate the significance of this observation, we examined the functional status of AMPK. Since AMPK is the substrate of LKB1 that negatively regulates mTOR activity via TSC1/2^{24, 16}, the level of phospho-AMPK(Thr172) can be used as an indicator of its activity. In the AMLs, the expression of phospho-AMPK was at least equivalent to that of normal kidney (cases 1 and 4), if not stronger (cases 2 and 3) (Figure 4B). This indicates that AMPK was active in the tumors, and therefore should suppress mTOR activity. Since the opposite was observed, we can conclude that the LKB1/AMPK pathway was not the

primary event leading to mTOR activation in these lesions. Functioning through ERK phosphorylation of TSC2, the Ras/MAPK pathway can also modulate mTOR²⁵. However, ERK phosphorylation was found to be elevated in only 1 of 4 AMLs (i.e., case 2) (Figure 4B). Collectively, the Western blotting analyses suggest the loss of TSC2 as a common underlying mechanism for mTOR activation in sporadic AMLs.

PEComas exhibit aberrant mTOR activity

Next, we determined if the broader group of PEComas, of which AML represent a subset, shared a similar functional aberration in mTOR signaling. Fifteen cases of PEComas were chosen among 26 previously reported cases based on availability of formalin-fixed, paraffin-embedded tumor materials²⁶. The clinicopathologic features of these tumors were summarized in a recent publication²⁶. Of note, none of these cases had a history of TSC; this is consistent with the observation that less than 10% of non-AML, non-LAM PEComas are associated with TSC. Here, we examined the expression of phospho-p70S6K and phospho-AKT by IHC. Our results show that all 15 tumors exhibited immunoreactivity of varying degrees to the anti-phospho-p70S6K antibody. Eight tumors had diffusely weak staining (2+) while 3 had focally strong (3+) and 4 had diffusely strong (4+) immunoreactivities (Figure 5). All but one PEComa failed to stain (0+) with anti-phospho-AKT antibody, and one had a few focal areas of weak staining (1+). Due to the lack of fresh tissue, we were unable to confirm these findings by Western blot analysis. Nonetheless, these results suggest that the PEComas behave similarly to AMLs with respect to mTOR signaling. Increased p70S6K and decreased AKT activities in these tumors would support the loss of TSC1/2 function as the underlying mechanism but we cannot exclude other mechanisms of mTOR activation.

Discussion

The pathogenesis of AMLs has intrigued pathologists for many years. Genetic analyses of sporadic AMLs have demonstrated that these tumors are clonally derived, but the studies have not provided consistent results with respect to an underlying genetic mechanism^{27–29}. Trisomy 7 has been independently reported in sporadic AMLs. Its significance remains uncertain given that non-neoplastic renal tissues often exhibit the same karyotypic change^{30–33}. The association between AML and TSC has led several investigators to examine alterations at the *TSC1* and *TSC2* loci, 9q34 and 16p13, respectively. Henske et al. found LOH on 16p13 in 5 of 8 TSC-associated AMLs and 3 of 29 sporadic AMLs⁸. Similarly, Smolarek et al. reported *TSC2* LOH in 7 of 13 AMLs associated with sporadic LAM⁹. In two reports by Martignoni et al., LOH on 16p was found in 2 of 4 cases of epithelioid AMLs in non-TSC patients^{10,11}. However, Kattar et al. was unable to detect loss of 16p in 11 sporadic AMLs using comparative genomic hybridization³⁴. Nonetheless, these studies suggest the possible role of *TSC2* in sporadic AMLs unrelated to TSC or LAM, but the functional significance of this pathway has not been determined.

In this study, we discovered that sporadic AMLs uniformly exhibit activation of the mTOR cascade. This is similar to the findings reported for TSC and LAM-associated AMLs suggesting a common pathogenetic mechanism^{22,35,36}. We found robust expression of phospho-p70S6K and lack of phospho-AKT expression in all 15 cases examined by immunohistochemistry. This implies that sporadic AMLs represent a homogenous class of neoplasm. In the subset of four lesions where fresh tissues were obtained, our results indicate the loss of *TSC2* as the primary event in their pathogenesis. This is in keeping with the findings of *TSC2* LOH in genetic analyses of sporadic AMLs. The apparent lack of *TSC1* involvement is unclear since patients with *TSC1*-related tuberous sclerosis also develop AMLs. However, it has been reported that *TSC2* is more commonly affected in non-familial cases of TSC³⁷. Further, genotype-phenotype studies suggest that *TSC2*-related pathologies are more severe than the *TSC1* cases³⁷. Thus,

we speculate that *TSC2*-affected AMLs are more likely to be detected and/or clinically symptomatic than *TSC1*-affected AMLs, although larger studies are needed to confirm our findings.

The observed increase in p70S6K and PHAS1 phosphorylation along with reduced AKT phosphorylation represents the molecular signature of cells lacking a functional *TSC1/2* complex (Figure 1C). This is distinct from the pattern observed following the loss of *PTEN*, which results in increased AKT phosphorylation (Figures 1B). The unique suppression of AKT activity observed in the *TSC2*-null state is secondary to the negative feedback of p70S6K on IRS1 and insulin signaling²¹. It has been suggested that the reduced AKT activity in *TSC*-related tumors may be relevant to their benign behavior¹⁹. Conversely, AKT activation in the setting of *PTEN* deficiency has been commonly observed in human malignancies³⁸. In this study, we also observed an inverse relationship between *TSC2* and *PTEN* expression not previously reported. Despite increased *PTEN* expression in the AMLs, mTOR activity remains elevated due to the loss of *TSC2*. This up-regulation of *PTEN* may represent a compensatory mechanism of the cell to normalize its elevated mTOR activity. Serving as a phosphatase, *PTEN* antagonizes the activity of PI3K¹⁴ and could further suppress AKT function in these lesions. Currently, *PTEN* activity and the mechanism of its regulation and expression in AMLs have not been elucidated.

Multiple pathways converge on mTOR to regulate its function. Besides *TSC1/2* and *PTEN*, the tumor suppressor proteins, LKB1 and NF1, inhibit mTOR through their influence on AMPK and the Ras/MAPK pathways, respectively^{16,17,25}. Based on the levels of phosphorylated AMPK and ERK, we failed to find evidence that either of these signaling cascades could account for the mTOR activities found in the AMLs. Instead, our collective data favor *TSC2* loss as the primary mechanism of mTOR dysregulation.

Non-AML PEComas share common histologic and immunophenotypic characteristics with AMLs. Their relative scarcity provides few clues to their pathogenesis. A recent review of the literature found that a minority (<10%) of soft tissue and gynecologic PEComas are associated with *TSC*²⁶, thus providing a plausible link to an underlying genetic pathway. This is supported by a comparative genomic hybridization study by Pan et al. showing multiple recurrent chromosomal alterations including the loss of 16p¹². In this study, we show for the first time that p70S6K is phosphorylated while AKT is non-phosphorylated in all cases examined except for one lesion with limited phospho-AKT expression. Once again, these results point to the involvement of the *TSC*/mTOR pathway in non-AML, non-LAM PEComas. However, given the lack of fresh tissues, we were unable to perform immunoblot analyses to confirm or characterize the mTOR pathway in these tumors. Compared to the AMLs, the immunostaining results were more variable, and this may be due to technical differences in the fixation process, the age and storage of the specimens, and/or a reflection of the involvement of alternative molecular pathways. Further biochemical and genetic analyses are necessary to define the mechanisms of mTOR activation in these tumors. Nonetheless, the highly consistent finding of p70S6K phosphorylation provides a novel target for therapy.

Besides surgery, there is no effective medical treatment for AMLs or PEComas. The results of this study suggest that mTOR hyperactivity may contribute to tumor growth and progression. Rapamycin is a potent and specific inhibitor of mTOR by acting through a cellular chaperone, FKBP12³⁹. It is approved by the FDA for immunosuppression therapy following renal transplant⁴⁰. Pre-clinical studies in animal models of *TSC* have shown significant *in vivo* response to rapamycin and its derivatives^{41, 42}. Results in *TSC* and LAM patients are pending. These drugs are also being tested in a wide array of human malignancies and have been recently approved for use in renal cell carcinomas and acute myeloid leukemia^{43,44}. Given the relative

low toxicity profile, rapamycin and its derivatives may provide therapeutic benefits in the treatment of AMLs and non-renal PEGomas.

The origin of PEGomas remains unknown and controversial. To date, no progenitor cell has been identified and no normal counterpart displaying the dual phenotype of smooth muscle and melanocytic differentiation has been found. The involvement of the TSC pathway provides possible clues to this dilemma. It has been proposed that B-raf activity in cells lacking TSC2 may play a role in differentiation but the functional link and its mechanism remain unclear⁴⁵. Alternatively, we have previously reported that the TSC pathway negatively regulates the Wnt/ β -catenin pathway by promoting the activity of its degradation complex⁴⁶. β -catenin is known to regulate transcription of crucial genes involved in proliferation and differentiation. Future studies are aimed at analyzing the contributions of these pathways in the pathogenesis of PEGomas.

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Figure 1.

The TSC/mTOR signaling pathway. A) PI3K-dependent activation of AKT leads to the inhibition of TSC2 activity resulting in an increase in Rheb-GTP level and downstream activation of mTOR, which in turn phosphorylates p70S6K and PHAS1 to promote protein synthesis. LKB1 phosphorylates AMPK to enhance TSC1/2 inhibition of mTOR. Acting down-stream of Ras, ERK and RSK phosphorylates TSC2 to repress its GAP activity. In a negative feedback loop, p70S6K phosphorylates IRS1 to reduce insulin sensitivity and AKT activity. Tumor suppressor genes that inhibit mTOR activity are in bold. B, C) Simplified versions of the pathway to highlight the functional consequences of the loss of PTEN (B) and TSC1/2 (C) are shown to illustrate the unique difference in AKT activity of the two scenarios. Arrows indicate relative change in activities.

Figure 2. Immunophenotype of a TSC2-related AML. The loss of TSC2 function results in A) up-regulation of mTOR as illustrated by increased levels of phospho-p70S6K(Thr389) expression in the tumor (T) compared to adjacent normal kidney (N), and B) down-regulation of AKT as shown by the lack of phospho-AKT(Ser473) immunoreactivity in the tumor (T). C) In contrast, prostate carcinoma, which frequently undergo PTEN loss, expressed high levels of phospho-AKT(Ser473) compared with adjacent atrophic gland (N). Magnification 400x.

A

Case 1

Case 2

p-p70S6K
Thr(389)

Figure 3. Immunohistochemical analysis of the mTOR pathway in sporadic AMLs. Examples of two sporadic, non-TSC AMLs analyzed by immunohistochemistry for A) phospho-p70S6K, B) phospho-S6, and C) phospho-AKT. T, tumor; N, normal kidney. Magnification: for A and B, top panel: 100x; bottom panel: 400x; for C, 200x.

Figure 4.

Biochemical analysis of the mTOR pathway in sporadic AMLs. Four independent, non-TSC AMLs were analyzed by Western blotting using antibodies indicated. Panel A highlights the targets of mTOR: p70S6K, ribosomal S6, and PHASI, as well as AKT. Panel B illustrates the up-stream negative regulators of mTOR: TSC1, TSC2, PTEN, LKB1, AMPK, and ERK. Each sample is represented by normal kidney (NK), and tumor (AML). Actin serves as loading control.

p-p70S6K Thr(389)

Case 1

Case 2

Figure 5. Immunohistochemical analysis of p70S6K in PEComas. Two examples of PEComas immunostained for phospho-p70S6K. Magnification: 100x (top) and 400x (bottom).