Tuberous sclerosis complex: Hamartin and tuberin expression in renal cysts and its discordant expression in renal neoplasms


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A B S T R A C T

Tuberous sclerosis complex (TSC) results from mutation of TSC1 or TSC2 that encode for hamartin and tuberin. It affects the kidneys often in advance of extra-renal stigmata. We studied 14 TSC cases, and 4 possible TSC cases with multiple angiomyolipomas (AMLs) for hamartin and tuberin protein expression to determine if the staining profile could predict mutation status or likelihood of TSC with renal-limited disease. The 18 cases included 15 nephrectomies and 1 section of 6 TSC-associated renal cell carcinomas (RCC). Controls included the non-neoplastic kidney in 5 tumor nephrectomies, 4 sporadic cases of AML and 6 clear cell RCCs. In the 14 TSC cases, 9 had AMLs, 9 had RCCs, 5 had polycystic kidney disease and 8 had eosinophilic cysts (EC) lined by large eosinophilic cells. The controls and study cases showed luminal staining of proximal tubules (PT) and periluminal membrane staining in distal tubules/collection ducts for hamartin and cytoplasmic staining for tuberin. Eosinophilic cysts had a luminal PT-like stain with hamartin and a cytoplasmic reaction for tuberin. Hamartin stained myoid cells in all AMLs. Tuberin was negative in all but 1AML, an epithelioid AML. All but 1 RCC were positive for tuberin; 13 RCCs (7 TSC/6 non-TSC) were negative for hamartin and 4 showed a weak reaction. We conclude that the ECs of TSC are proximal tubule-derived. The hamartin and tuberin staining profiles of AMLs and most RCCs are reciprocal precluding prediction of the mutation in TSC, and fail to predict if a patient with multifocal AML has TSC.

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1. Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder that affects 1 in 6000 people [1,2]. It results from mutation of 1 of 2 genes, TSC1 or TSC2, that encode for hamartin and tuberin, respectively, and has 95% penetrance but highly variable clinical expression and severity [1–4]. TSC is less common than TSC2 accounting for 29% of cases, while TSC2 is associated with a more severe clinical phenotype [2].

Tuberous sclerosis complex is characterized by neoplasms and cysts that affect multiple organs [1,2]. Renal involvement occurs in 60–80% and is clinically significant in 45%. It consists of angiomyolipomas (AML), cysts and polycystic kidney disease (PKD), and rarely, renal cell carcinoma (RCC) and oncocyotma [5–12]. The renal lesions may occur singly or in combination, are often multifocal and bilateral, and may precede other stigmata of TSC [1,2,5,6]. The diagnosis of TSC can be challenging since the profile of organ
involvement is diverse, and 65% of cases represent a new mutation so a positive family history is often lacking [1,2].

The clinical criteria for a diagnosis of TSC have evolved over the past few decades. Gomez in 1991 proposed a hierarchy of clinical and imaging features clustered into three categories; definitive, presumptive and suspect [13]. With the Gomez Criteria, the presence of multiple AMLs in a single kidney was regarded as definitive of TSC. The diagnostic criteria were made more stringent in 1998 and updated in the 2012 International Tuberous Sclerosis Complex Consensus Conference [14]. A definitive diagnosis of TSC now requires 2 different major lesions, or 1 major and 2 minor lesions, rather than multiple lesions of the same type [14]. Even with 2 major features to support a clinical diagnosis of TSC, genetic testing will only identify a mutation in 85% of patients [2].

Since renal involvement may precede other stigmata of TSC and parents with only AMLs may have children severely affected by TSC, diagnostic quandaries arise when multifocal renal AMLs are identified [15]. This study evaluates the immunohistochemical staining profiles for hamartin and tuberin in TSC to determine their utility as a surrogate marker for the underlying genetic mutation, especially relevant in the cases of possible TSC where multifocal renal AMLs are present as the sole clinical finding.

2. Material and methods

Eighteen cases of TSC (14 cases) or possible TSC (4 cases) were studied. The TSC cases fulfilled criteria of the 2012 International Tuberous Sclerosis Complex Consensus Conference [14]. The possible TSC cases contained multifocal AMLs which satisfied the 1991 Gomez criteria for TSC [13]. The cases were obtained from multiple institutions; Indiana University, Indianapolis, IN, The University of Arkansas for Medical Sciences, Little Rock, AR, Louisiana State University, Shreveport, LA, Mayo Clinic, Rochester, MN, Cleveland Clinic, Cleveland, OH, William Beaumont Hospital, Royal Oak, MI, El Camino Hospital, Mountain View, CA, Stanford University, Stanford, CA, and Lille University Hospitals, Lille, France.

Immunoperoxidase stains for hamartin, C-2 monoclonal IgG2 mapping to N-terminus of hamartin of human origin (1:100 dilution) and tuberin, N-19 rabbit polyclonal IgG mapping to the N-terminus of tuberin of human origin (1:100 dilution), Sanus Cruz Biotechnology, were performed. The materials available for review consisted of 15 nephrectomies or partial nephrectomies from twelve cases (3 were bilateral) and a single section of a RCC in 6 cases, for a total of 21 specimens. The non-neoplastic kidney from 5 nephrectomies performed for renal cell carcinoma, 4 cases of sporadic AML and 6 cases of sporadic clear cell RCC in patients served as immunoperoxidase stain controls. Demographic and clinical information was available on all cases. This study is IRB approved.

Immunoperoxidase staining was semi-quantitatively scored as 0–3+ as follows:

0−no staining

1+—staining clearly above the background negative cells involving fewer than 25% of the cells of interest

2+—prominent staining of a large fraction of the cells, 25–50%, or all of the cells of interest, but less than the strongest positive controls

3+—diffuse staining in >50% of the cells of interest, equal to the strongest positive controls

3. Definitions

For purposes of this study the following definitions were employed.

Cyst—an epithelial lined structure grossly visible in a nephrectomy specimen or on a glass slide.

Table 1
Tuberous sclerosis-related pathology findings.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/sex</th>
<th>Cysts</th>
<th>Angiomyolipoma</th>
<th>RCC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Angiomyolipoma</td>
<td>Macro</td>
</tr>
<tr>
<td>PKD</td>
<td>EC</td>
<td>EMC</td>
<td></td>
<td></td>
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<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>1w/F</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>38y/F</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>58y/F</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>21y/F</td>
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<td>69y/F</td>
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<td></td>
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<td>5</td>
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<td>12</td>
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</table>

AML—Angiomyolipoma; Macro—Macroscopic; Micro—Microscopic; AMLEC—Angiomyolipoma with epithelial cyst; AMLoss—Angiomyolipomatosis; PKD—Polycystic kidney disease; EC—Eosinophilic cyst; EMC—Eosinophilic micro-cyst; RCC—Renal cell carcinoma; A—Eosinophilic-microcystic RCC; B—Renal angioadenomyomatous tumor; C—Chromophobe cell RCC.

n/a—Not applicable because one section was reviewed for this study.

Eosinophilic micro-cyst—an ectatic tubule not grossly visible in a nephrectomy specimen or on a glass slide, lined by eosinophilic cells with prominent nucleus and having larger cytoplasmic volume and luminal diameter than the adjacent normal proximal tubules.

Eosinophilic cyst—an epithelial-lined structure grossly visible in a nephrectomy specimen or on a glass slide lined by enlarged eosinophilic cells similar to the eosinophilic microcysts.

Poly-cystic kidney disease—a kidney diffusely transformed by cysts. Its overall size may be smaller or larger than normal.

Angiomyolipoma, macroscopic—a grossly visible angiomyolipoma. It may be a classic tri-phasic tumor containing lipid-rich cells, myoid cells and abnormal arteries, or may be lipid-rich cell or myoid cell predominant.

Angiomyolipoma with an epithelial-lined cyst (AMLEC)—an angiomyolipoma variant consisting of a myoid predominant AML that contains an epithelial-lined cyst surrounded by a cellular “cambium” layer interposed between the myoid cells and the cyst [16].

Angiomyolipomatosis—an ill-defined circumscribed interstitial proliferation of angiomyolipomatous tissue consisting of myoid cells, lipid-rich cells, or both, present as individual cells or forming cords of cells. Abnormal arteries are not present.

4. Results

4.1. Definition of groups

Twenty-one specimens from 18 patients were reviewed. The findings were similar for each side in patients in which both kidneys were examined so the results will be presented as number of patients rather than number of specimens examined. The cases were divided into two groups (Table 1). Group 1 consists of 14 TSC cases that satisfied the criteria of the 2012 International Tuberous
Sclerosis Complex Consensus Conference [14]. Group 2 consisted of 4 possible TSC cases that had multifocal AMLs but no extrarenal stigmata of TSC that satisfied the 1991 Gomez criteria for TSC [13].

4.2. Group 1

Group 1 consisted of 2 children and 12 adults. The ages of the 2 children were 1 week and 11 years. The ages of the adults ranged from 21 to 58 years with an average of 37 years. In 12 nephrectomies multifocal AMLs were present in 9 cases that ranged from macroscopic tumors (7 cases) to microscopic AML (6 cases) (Fig. 1A). The composition of the AMLs varied from predominately lipid-rich cells, to predominately myoid cells, to triphasic. Two cases demonstrated AMLosis, characterized by ill-defined proliferations of myoid cells and lipid-rich cells that insinuated between normal or atrophic nephron elements (Fig. 1B). Three patients had AMLECS.

Renal cell carcinomas were present in 9 patients (Table 1). Six cases were previously reported in detail by 3 authors (AS, XL and JM) [17]. The histology of the RCCs varied; 3 “types” of RCCs were identified. Two RCCs resembled the so-called renal angiomyoadenameomatous tumor (RAT), a tumor characterized by nests of large cells with clear cytoplasm traversed by bundles of smooth muscle. Three RCCs resembled sporadic cases of chromophobe cell RCC. Five RCCs were composed of large eosinophilic focally vacuolated cells with large vesicular nuclei that exhibited architectural variability, occasionally solid, but often cystic. One patient had multiple and bilateral macroscopic RCCs and numerous microscopic RCCs and 2 patients had 2 small RCCs (3 mm to 1 cm). These eosinophilic-cystic RCCs would qualify as RCC, unclassified by the 2004 WHO and the 2013 ISUP classifications [18,19].

Eosinophilic cysts (EC) and eosinophilic microcysts (EMC) were present in 5 and 8 patients, respectively, associated with cysts having flattened to low cuboidal epithelium. The ECs and EMCs were lined by large cells with dense eosinophilic, occasionally vacuolated cytoplasm and vesicular nuclei (Fig. 2A). Although a single layer of eosinophilic cells was most common, occasionally cell stratification was present. Five patients had numerous bilateral cysts consistent with PKD (Fig. 2B). The cysts included both ECs and EMCs, and cysts lined by flattened or cuboidal epithelium. Six of 8 cases with ECs and/or EMCs had macroscopic AMLs and 3 of 8 also had RCCs.

4.3. Group 2

Group 2 consisted of 4 patients with multifocal, or multifocal and bilateral AMLs. Their average age was 57 years, 20 years older than cases in Group 1 (Table 1). Two patients with the largest AMLs, both 9 cm, presented with perinephric hemorrhage. The AMLs ranged from macroscopic to microscopic (Fig. 3). One case also contained 2 AMLECs. AMLosis was not present, and by definition, PKD, EC and EMC were not present.
4.4. Hamartin and Tuberin Immunohistochemistry

Immunohistochemical stains for hamartin and tuberin were performed on 5 control nephrectomies, 4 sporadic AMLs (single tumor), 6CC-RCCs and on 1–3 blocks from cases in Group 1 and Group 2. The cases were examined for hamartin and tuberin staining of the various normal nephron components, and the cysts, microcysts and neoplasms, if present.

Control nephrectomies showed hamartin and tuberin staining in all nephron structures, glomeruli, proximal tubules (PT) and distal tubules (DT), and in collecting ducts (CD) (Fig. 4A and B). The glomerular staining was weak (1+), possibly because the cytoplasmic volume of glomerular cells is much less than tubular cells. Parietal epithelial cells, podocytes, mesangium cells, endothelialial cells stained positive in glomeruli of all cases. The vascular endothelium of arterioles and arteries, and the smooth muscle cells of arterioles, arteries, veins and collecting system muscularis were also positive. The pattern of tubular staining differed between hamartin and tuberin. Hamartin demonstrated a 2–3+ distinct brush border-like luminal staining of PT and a peripheral cell membrane pattern in DT and CD with weak cytoplasmic staining (Fig. 4A). Conversely, tuberin showed a 3+ diffuse cytoplasmic reaction in all tubular segments (Fig. 4B).

The normal nephrons of 11 cases from Group 1 and 4 cases of Group 2 showed an identical staining pattern for hamartin and tuberin as the controls. Three RCCs from Group 1 lacked an internal control of normal kidney for evaluation. The ECs and EMCs of Group 1 stained for both hamartin and tuberin (Table 2) (Fig. 5A–C). The hamartin stain pattern was apical in most ECs and in all EMCs, similar to the staining of control proximal tubules. Tuberin showed a diffuse cytoplasmic reaction. Cysts with a flat atrophic epithelial lining were usually negative for both hamartin and tuberin.

Hamartin stained all 22 macroscopic AMLs and all microscopic AMLs in which myoid cells were present (Table 2) (Fig. 6A). The staining pattern ranged from 3+ diffuse and strong, to 2+ moderate focal staining similar to the focal staining with HMB-45 characteristically observed in AMLs. The lipid-rich cells of AMLs appeared negative, therefore, lipid-rich cell predominant AMLs were largely or completely negative. The 2 cases with interstitial AMLOsis showed extensive interstitial hamartin positive myoid cells. All 4 of the AMLECs showed 2–3+ hamartin staining of the cyst epithelium, the cambium layer cells and the AML myoid cells. Hamartin did not show a luminal or peripheral cell membrane pattern of normal PT or DT/CD, respectively. Hamartin also stained 2–3+ the multifocal AMLs of Group 2 that contained myoid cells and the 4 sporadic cases of AML.

Tuberin staining was completely negative in 21 of 22 macroscopic AMLs, all microscopic AMLs and in AMLOsis (Fig. 6B). The one macroscopic AML positive (3+) for tuberin was an epithelioid AML, the only one in this study. All 4 of the AMLECS showed 2–3+ tuberin staining of the cyst epithelium while the cambium layer cells and myoid cells stained in 1 of 4 cases. None of the multifocal AMLs of Group 2 or the 4 sporadic cases of AML showed tuberin staining.

Nine TSC cases with 11 RCCs of the three types were studied for hamartin and tuberin. All 11 RCCs demonstrated a diffuse 2–3+ reaction for tuberin (Table 2) (Fig. 7A). Their supporting microvasculature, however, was negative for tuberin. The smooth muscle within the 2 RAT tumors was tuberin negative. Tuberin was also positive (1–2+) in 5 of 6 control CC-RCC.

Seven RCCs were negative for hamartin (Fig. 7B). Four RCCs (2/3 Chromophobe, 1/2 RAT, 1/4 eosinophilic-microcystic), however, showed a 1+ reaction for hamartin. The supporting microvasculature in all RCCs stained strongly for hamartin, and the smooth muscle within the 2 RAT tumors was also strongly positive (3+). The 6CC-RCCs were negative for hamartin. However, their supporting microvasculature was positive.

Five patients had both RCCs and AMLs that, in general, showed a reciprocal tumor staining for hamartin and tuberin (Fig. 8A–C). Three patients showed positive hamartin/negative tuberin in their AMLs, with negative hamartin/positive tuberin in their RCCs. The 2
other patients also showed positive hamartin/negative tuberin in their AMLs, but had weak (1+) positive hamartin/positive tuberin in their RCCs.

5. Discussion

Tuberous sclerosis complex is a disorder of cellular migration, proliferation and differentiation leading to cysts and neoplasms that affect multiple organs, most commonly skin, central nervous system and kidney [1, 2]. Tuberous sclerosis complex results from a mutation of TSC1 or TSC2, that encode for hamartin and tuberin, respectively [3, 4]. More than 300 allelic variants of TSC1 and more than 1000 allelic variants of TSC2 have been described. Approximately 65% of TSC1 represent a de-novo mutation, so family history is often lacking [1–5]. Mutations involving TSC1 tend to be small deletions or insertions and nonsense mutations, while TSC2 may have large deletions or rearrangements [20]. Approximately 2% of TSC2 patients have large mutations affecting both TSC2 and PKD1, an adjacent gene on chromosome 16, resulting in the TSC2/PKD1 contiguous gene syndrome (CGS) and early onset PKD, often prior to other stigmata of TSC [8, 10–12]. The lesions of TSC are believed to follow the classic "Knudson model" where a single allelic germline mutation of TSC1 or TSC2 is coupled with a somatic mutation to the other allele leading to loss of function [1, 2].

The products of TSC1 and TSC2, hamartin and tuberin, are multifunctional proteins that form a cooperative complex [21–25]. The clinical similarity between patients with TSC1 or TSC2 mutations supports the notion that mutations in either gene affect the function of the TSC protein complex. In normal cells, the TSC complex negatively regulates mammalian target of rapamycin (mTOR), a component of the P13K/AKT/mTOR pathway, through inhibition of GTP-binding protein Rheb (Ras homolog enriched in brain) [21–25]. Loss of the TSC complex leads to unregulated Rheb activation of mTORC1, a complex containing mTOR, to stimulate downstream pathways involved in cell growth and proliferation via phosphorylation of ribosomal protein S6 kinase beta 1 and eukaryotic initiation factor 4E-binding protein-1. Evidence also suggests that hamartin and tuberin have individual, non-TSC complex related activities [24, 25].

Renal AMLs are the most common renal abnormality in TSC. Like their sporadic counterparts, they show considerable histologic variation with lipid-rich cell or myoid cell predominance, show an epithelioid or oncocytoma-like phenotype and may contain an epithelial cyst (AMLEC). We describe herein an interstitial AML proliferation, referred to as angiomyolipomatosis, composed of individual cells or slender cords of cells that infiltrate around nephron elements. It lacks the circumscription of microscopic AMLs, and may replace the majority of the renal parenchyma without producing a grossly visible lesion.

The presence of multiple AMLs has historically raised concern of a forme fruste of TSC (Gomez criteria) when a family history is lacking since a parent with a single AML may have a child severely affected by TSC [15]. Multifocal AMLs in patients with TSC when compared to those without TSC are more often large and symptomatic, and AMLEC, EpAML and microscopic AMLs with multiple elements rather than myoid cells alone, are more common in TSC [26, 27]. Although a combination of EpAML, microscopic AMLs and AMLEC was 100% predictive of TSC in one study, only 3 of 16 cases showed these features [27]. Genetic confirmation or satisfying the Tuberous Sclerosis Consensus Conference criteria are the only means to definitively diagnosis TSC; some cases may never be identified.

We stained the spectrum of TSC renal lesions for hamartin and tuberin to determine if the staining profile could serve as a surrogate marker for the TSC1 or TSC2 mutation present, or imply the presence of TSC in a patient with multiple AMLs without extrarenal stigmata. Although we lack genetic data, in a series of 14 TSC patients a couple of TSC1 mutations could be present, although, most would likely be TSC2. We found that hamartin and tuberin expression of control kidneys was identical to the staining pattern of TSC and possible TSC cases. Although only a single functional allele of one TSC gene was present, no difference in staining intensity of normal nephrons between TSC and controls was identified to suggest the underlying mutation.

We found that the cellular localization of hamartin and tuberin in normal tissues differed. Hamartin demonstrated a luminal brush border-like reaction in PT, and an apical and peripheral cell membrane reaction in DT/CD. The apical and peripheral staining patterns may reflect hamartin’s interaction with the e-zrin-radixin-moesin family of activating proteins involved in cell adhesion [28]. In contrast, tuberin demonstrated a cytoplasmic reaction consistent with its localization in the Golgi apparatus and its role in regulation of cytoplasmic vesicle transport to the cell membrane [28]. Autopsy studies of TSC and non-TSC patients have found that renal tubules stain for both proteins but differed from our results showing distal tubule accentuation for both without a brush border-like luminal

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Table 2

<table>
<thead>
<tr>
<th>Hamartin and Tuberin Immunohistochemistry.</th>
<th>Hamartin Positive (Intensity)</th>
<th>Tuberin Positive (Intensity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberous Sclerosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophilic cysts and microcysts (# cases)</td>
<td>8 of 8</td>
<td>7 of 7</td>
</tr>
<tr>
<td>Angiomyolipomas (# tumors)</td>
<td>22 of 22 (2–3+)</td>
<td>1+ of 22 (3+)</td>
</tr>
<tr>
<td>AMLEC (# tumors)</td>
<td>4 of 4</td>
<td>4 of 4</td>
</tr>
<tr>
<td>Epithelium</td>
<td>4 of 4</td>
<td>1 of 4</td>
</tr>
<tr>
<td>Cambium, Smooth muscle</td>
<td></td>
<td></td>
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<tr>
<td>Renal cell carcinomas (# tumors)</td>
<td>1 of 4 (1+)</td>
<td>4 of 4 (2–3+)</td>
</tr>
<tr>
<td>Eosinophilic-microcystic RCC</td>
<td></td>
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<tr>
<td>Renal angioadenomyomatous tumor</td>
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<td></td>
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<tr>
<td>Smooth muscle stroma</td>
<td>2 of 2 (3+)</td>
<td>2 of 2 (2+)</td>
</tr>
<tr>
<td>Chromophobe RCC</td>
<td>2 of 3 (1+)</td>
<td>3 of 3 (2–3+)</td>
</tr>
<tr>
<td>Total RCCs</td>
<td>4 of 9 (1+</td>
<td>9 of 9 (2–3+)</td>
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<td>Possible Tuberous Sclerosis (# cases)</td>
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<tr>
<td>Multifocal AML</td>
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<td>Non-tuberous Sclerosis (# cases)</td>
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<td>Single AML</td>
<td>4 of 4 (2+)</td>
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<tr>
<td>Clear cell RCC</td>
<td>0 of 6</td>
<td>5 of 6 (1–2+)</td>
</tr>
</tbody>
</table>

AMLEC—angiomyolipoma with epithelial cysts; RCC—renal cell carcinoma.

* Epithelial AML.
staining of PT for hamartin [29,30]. Post mortem autolysis affecting antigenicity may explain this difference.

The PT brush border-like staining of EC and EMC with hamartin support PT derivation. This fits with the histology of EMC that resembles PT and their characteristic location in the cortical labyrinth predominately populated by PT. One might predict that the functionality of hamartin or tuberin would be affected in the cyst epithelium depending upon the mutation due to a second hit to the normal allele. However, the protein expression with the antibodies employed, appeared unaltered. Although haploinsufficiency for TSC1 and TSC2 has been shown to be sufficient for cyst formation in rat (Eker) and mouse models of renal cyst formation, mutation of the normal allele in TSC may not be required for cyst formation in humans. The positive staining may represent wide-type protein [31,32].

We unexpectedly found reciprocal expression of hamartin and tuberin between AMLs and RCCs, but similar staining within the types of RCCs. Hamartin was ubiquitously expressed in AMLs from TSC, possible-TSC cases and sporadic non-TSC cases. Conversely, tuberin was negative in all AMLs except 1 of our only EpAML, of note since tuberin expression is a feature of epithelial differentiation in RCCs. Plank, et al. in their IH study of multiple AMLs in two TSC patients reported hamartin negative/tuberin positive AML staining in a TSC1 patient and hamartin positive/tuberin negative AML staining in a TSC2 patient, suggesting that loss of IH staining correlated with the gene mutation [33]. By extrapolation, these findings could imply the presence of a TSC2 mutation in our TSC patients with AMLs. However, since we had 5 patients with hamartin positive/tuberin negative AMLs who had concurrent tuberin positive RCCs that showed no (3 cases) or weak hamartin staining (2 cases), this postulate appears to be invalid in our cases.

The expression of hamartin or tuberin in TSC neoplasms appears independent of the underlying mutated gene in a cell differentiation specific manner. The myoid cells and arteries in AMLs, smooth

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**Fig. 5.** (A) Eosinophilic microcyst. This is an eosinophilic microcyst in a TSC patient. (B) Eosinophilic microcyst. The eosinophilic microcyst in (A) is stained for hamartin. Note the brush border staining reaction of equal intensity as the adjacent normal proximal tubules. Immunoperoxidase stain for hamartin. (C) Eosinophilic microcyst. The eosinophilic microcyst in (A) is stained for tuberin. Note the diffuse cytoplasmic staining reaction of equal intensity as the adjacent normal tubules. Immunoperoxidase stain for tuberin.

**Fig. 6.** (A) Angiomyolipoma. The macroscopic myoid cell predominant angiomyolipoma at the top shows diffuse intense hamartin staining. The cortex below serves as a positive internal control. Immunoperoxidase stain for hamartin. (B) Angiomyolipoma. This field is comparable to (A). It shows no tuberin staining of the macroscopic angiomyolipoma on the top. The cortex below serves as a positive internal control. Immunoperoxidase stain for tuberin.
Fig. 7. (A) Renal cell carcinoma. This eosinophilic-cystic renal cell carcinoma is positive for tuberin while its supporting capillaries are negative. Immunoperoxidase stain for tuberin. (B) Renal cell carcinoma. This eosinophilic-cystic renal cell carcinoma is negative for hamartin although its supporting capillaries are positive. Immunoperoxidase stain for hamartin.

Fig. 8. (A) Angiomyolipoma and renal cell carcinoma. This shows an angiomyolipoma at the top and an adjacent eosinophilic-cystic renal cell carcinoma at the bottom. Immunoperoxidase stain for hamartin. (B) Angiomyolipoma and renal cell carcinoma. This field is comparable to that in (A) but it is not as clear. (C) Angiomyolipoma and renal cell carcinoma. This field is comparable to (A) but it is not as clear. Immunoperoxidase stain for tuberin.

muscle in RAT tumors and supporting vasculature in RCCs solely express hamartin, while tuberin is strongly expressed in RCCs in TSC patients, in 5 of 6c-RCC cases and in 1 EpAML. This suggests an imbalance in expression may affect cellular differentiation in TSC tumorigenesis, consistent with reports showing participation of TSC-complex and mTORC1 in epithelial-mesenchymal and/or mesenchymal-epithelial transformation [34,35].

The lack of information about the mutation status of our cases represents the major limitation of this study. Knowledge of the specific TSC mutation(s) would enable additional assessment of downstream effects on protein expression and TSC-complex interaction. This could provide an explanation for the discordant expression of hamartin and tuberin observed in renal mesenchymal and epithelial neoplasms and their relationship to cellular differentiation.

In summary, we show that the IH staining profile for hamartin and tuberin in the normal kidney and EC and EMC of TSC patients is the same as controls despite the presence of only a single functional allele. The identical staining pattern of the TSC eosinophilic epithelium to PT implicates PT as the origin of the eosinophilic cysts. Despite the presumed presence of bi-allelic mutations in the renal tumors, the expression of hamartin and tuberin in our series of AMLs and RCCs is cell-type specific. Finally, the reciprocal staining profile of AML and RCCs in TSC and non-TSC does not permit implication of mutation status, nor allow determination if a patient with multifocal AML has TSC.

References


