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Original article

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

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ABSTRACT

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 peripheral membrane staining in distal tubules/collecting 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.

1. Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder that affects 1 in 6000 people [1,2]. It results from mutation of 1of 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]. TSC1 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 oncocytoma [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

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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), Santa 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.

Case	Age/sex	Cysts			Angiomyolipoma				RCC
		PKD	EC	EMC	Macro	Micro	AMLEC	AMLosis	
Group1									
1	1w/F	+	+	+					
2	38y/F	+	+	+	+	+		+	
3	58y/F	+	+	+	+				
4	21y/F			+	+	+	+		
5	30y/F			+	+		+		
6	23y/F	+	+	+		+			+ A × 2
7	36y/F	+	+	+	+	+		+	+ A – M/B
8	11y/F			+	+	+	+		+ A × 2
9	24y/F	n/a	n/a	n/a	n/a				+ A
10	38y/M	n/a	n/a	n/a	n/a				+ B
11	52y/M	n/a	n/a	n/a	+				+ B
12	25y/F	n/a	n/a	n/a	n/a				+ C
13	42y/F	n/a	n/a	+	+				+ C
14	58y/F	n/a	n/a	n/a	n/a				+ C
Group 2									
15	35y/F				+	+			
16	60y/F				+	+			
17	64y/M				+	+			
18	69y/F				+	+	+		
Total		5	5	9	12	9	4	2	9

AML—Angiomyolipoma; Macro—Macroscopic; Micro—Microscopic; AMLEC—Angiomyolipoma with epithelial cyst; AMLosis—Angiomyolipomatosis; PKD—Polycystic kidney disease; EC—Eosinophilic cyst; EMC—Eosinophilic microcyst; 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 microcyst—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.

Polycystic 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].

Angiomyolipoma, microscopic—a small, circumscribed, mm-sized or less AML, not grossly visible, usually lipid-rich or myoid cell predominant, often referred to in the literature as a "microhamartoma".

Angiomyolipomatosis—an ill-defined uncircumscribed 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