

Hamartin and Tuberin Expression in Human Tissues

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Tuberous sclerosis (TSC) is a bigenic autosomal dominant disease caused by mutations in one of two tumor-suppressor genes, TSC1 and TSC2, resulting in benign hamartomas and low grade neoplasms in multiple organs including brain, heart, kidney, and skin. We report the results of an immunohistochemical study of the expression of the TSC gene products, tuberin and hamartin, in multiple tissues obtained at autopsy from 12 non-TSC affected patients ranging in age from 20 weeks gestation to 8 years, and surgical specimens from some organs. Tuberin and hamartin are expressed and are colocalized in most tissues. Contrary to a previous report, immunostaining with our antisera detected hamartin in liver, small and large intestine, prostate, and testes. We did not detect significant developmental differences in tuberin or hamartin expression in comparable tissues from patients of different ages. Although tuberin and hamartin colocalize in most tissues and cell types, we provide data that hamartin is more abundantly expressed than tuberin in cells within some tissues including the distal nephron and a population of cells of the endocrine pancreas. These data support the hypothesis that hamartin and tuberin interact and may function together in many tissues where they are co-expressed, but also suggest that hamartin has a discrete and specialized function in certain cell types.

KEY WORDS: Hamartin, Immunohistochemistry, TSC1, TSC2, Tuberin, Tuberous sclerosis.

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Tuberous sclerosis (TSC) is an autosomal-dominant disorder characterized by hamartomatous malformations and low-grade neoplasms in various organs, including the brain, kidney, skin and heart. Common hamartomas associated with TSC include facial angiofibroma, cerebral cortical tuber, subependymal nodules, and giant-cell astrocytomas (SEGAs); retinal astrocytoma, cardiac rhabdomyoma, and renal angiomyolipoma (1, 2). Individuals with TSC are at high risk for developing renal tumors, including renal cell carcinoma and angiomyolipoma, early in life (3).

TSC has been linked to two separate loci. *TSC2* on Chromosome 16p13.3 produces a 5.5-kb transcript that encodes the ~180-kD protein tuberin; *TSC1* on 9q34 produces an 8.6-kb transcript that encodes the ~135-kD protein hamartin (4, 5). Tuberin contains a 58-amino acid region of homology to Rap1GAP and has specific GTPase-activating protein (GAP) activity *in vitro* for Rap1a (6). In addition, yeast two-hybrid analysis has identified a region of tuberin near the C-terminus and distinct from the region of GAP homology that binds to rabaptin-5 and stimulates GTP-Rab-5 hydrolysis and a reduction in the rate of fluid-phase endocytosis (7). Inactivation of the *TSC2* gene in cultured cells causes cytoplasmic localization and inactivation of the cyclin-dependent kinase inhibitor p27 (8). The exact role of these interactions—or disruption of them—in disease states is not yet known. In addition to data supporting a functional interaction between hamartin and tuberin (9), recent evidence suggests that hamartin regulates cell adhesion via its interaction with ezrin-radixin-moesin (ERM) proteins and activation of a Rho-mediated signaling pathway (10). Loss of heterozygosity at *TSC1* and *TSC2* chromosomal regions in TSC-associated hamartomas suggests that both proteins act as tumor suppressors (11, 12).

The tissue and cell-type distribution of the *TSC2* transcript and tuberin have been reported in organs of non-TSC-affected humans and rats (Tsc2; 13-16). In addition, the expression of hamartin in nor-

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TABLE 1. Description of Autopsy Tissues

Case #	Age	PMI (h)	Tissues Available*
1	20 wk of gestation	9	A, H, K, L, Lv, M, S, Th
2	21 wk of gestation	69	L, Lv, Pl, S, Th
3	25 wk of gestation	6	L, Lv, GI, H, S
4	25 wk of gestation	18	L, Lv, Pl, S, Th
5	1 d	10	A, GI, H, K, L, Lv, M, P, Pi, S, T, Th
6	4 d	72	GI, K, L, Lv, S, Th
7	1.5 mo	8	A, H, K, L, Lv, Ly, M, P, S, Thr
8	3 mo	14	GI, H, K, L, Lv, Ly, P, S, T
9	4 mo	0.5	Lv, M, P, Th, Thr
10	1 y	34	A, K, L, Lv, Ly, M, P, S, Th, Thr
11	4.5 y	50	A, H, K, L, Lv, Ly, S
12	8 y	12	GI, H, K, L, Lv, Pi, S, T

PMI = postmortem interval (approximate) from death to removal of organs/tissues.

* A = adrenal; GI = small and/or large intestine; H = heart (myocardium); K = kidney; L = lung; Lv = liver; Ly = lymph node; M = striated muscle; P = pancreas; Pi = pituitary; Pl = placenta; S = spleen; Th = thymus; Thr = thyroid; T = testis.

mal human tissues has recently been described (17). Here, we report the tissue and cell-type expression of hamartin as compared with that of tuberin in autopsy tissues from 12 non-TSC affected human, ranging in age from 20 weeks' gestation to 8 years, and from selected normal tissues obtained at surgery.

MATERIALS AND METHODS

Tissue

Paraffin-embedded autopsy tissue from 12 non-TSC-affected patients ranging in age from 20 weeks gestation to 8 years were used for this study. Cases were chosen such that a developmental study of the expression of tuberin and hamartin in comparable viscera could be performed on most tissues. Available autopsy tissues from each patient are listed in Table 1. In addition to autopsy tissues, all antisera were used for immunohistochemical staining of surgical specimens, including colon, kidney, liver, prostate, and pancreas. Description of the tissues studied and reasons for their removal are listed in Table 2.

Antisera

Polyclonal antisera recognizing amino acids (a.a.) 1463–1484 (α p3 tuberin antiserum) and 1787–1806 (α p1 tuberin antiserum) of tuberin (4) and a.a. 671–690 (671 hamartin antiserum) of hamartin (5) were prepared and affinity purified as described elsewhere (18). Polyclonal antiserum recognizing a.a. 1107–1130 (C-term hamartin antiserum) of hamartin, which has already been characterized (18), was used for immunoblots and immunohistochemistry on tissues from some cases.

Immunohistochemistry

Paraffin-embedded, formalin-fixed tissues removed at autopsy or surgery were sectioned at 6

μ m, dewaxed, and rehydrated through graded ethanols. Rehydrated sections were subjected to antigen retrieval in 0.1 M sodium citrate, pH 6.0, for 30 minutes at 100°C. Endogenous peroxidase activity was quenched by incubating with 3% hydrogen peroxide in phosphate-buffered saline for 20 minutes. Blocking was performed at room temperature with 5% bovine serum albumin/5% normal goat serum for 1 hour. Sections were incubated with dilute primary antisera overnight at room temperature. Three antisera were used at dilutions of 1/150–1/200 (\sim 6 μ g/mL), and C-term hamartin antiserum was used at a dilution of 1/500. These dilutions were chosen after a small pilot study performed to determine the optimal dilutions and conditions for producing qualitatively comparable staining intensities on most tissues with all antisera. Sections were subsequently incubated in anti-rabbit IgG antiserum (diluted 1/500 in blocking solution) for 1 hour at room temperature. Detection was performed using the ABC kit (Vectastain) and diaminobenzidine (3,3'-diaminobenzidine). Some tissues were counterstained with hematoxylin. Peptide preabsorption of the 671 hamartin and α p3 tuberin antisera, before their use, was performed in some immunohistochemical runs as a control for antibody specificity. In these studies, antisera and excess peptide (20 μ g of peptide per microgram of antisera) were diluted in 500 μ L of phosphate-buffered saline or blocking solution and incubated at 4°C for 2 hours before application to slides.

TABLE 2. Description of Surgical Specimens

Case #	Tissue	Diagnosis
13	Prostate	Prostatic adenocarcinoma
14	Pancreas	Serous microcystic adenoma
15	Prostate	Prostatic adenocarcinoma
16	Kidney	Renal cell carcinoma
17	Colon	Gunshot wound
18	Liver	Chronic hepatitis
19	Prostate	Prostatic adenocarcinoma

Immunoblotting

Autopsy tissues from two patients (tissues processed upon removal) and frozen autopsy tissues from one patient—including adrenal, brain, heart, kidney, liver, ovary, pituitary, skeletal muscle, stomach, spleen, and thyroid—were homogenized with a PT10/35 model Polytron (Brinkmann Instruments) in a buffer containing 0.32 M sucrose, 5 mM Hepes pH 7.4, 3 mM EGTA, 0.5 mM magnesium sulfate, and complete protease inhibitors (Roche, Indianapolis, IN). All tissues were collected within 10 to 15 hours of death. Total protein concentration was determined with the Bio-Rad Protein Assay (Bio-Rad Hercules, California). One hundred micrograms of total protein of each homogenate were resolved on 4%–15% polyacrylamide gels and transferred to PVDF for immunoblotting. Blots were incubated with C-term hamartin at a dilution of 1/2000 (0.5 $\mu\text{g}/\text{mL}$) for 5 hours. Immunolabeling was detected, after incubation with a secondary anti-rabbit IgG HRP-conjugated antibody for 1 hour, with Amersham electrochemiluminescence reagents (Amersham, Piscataway, New Jersey).

RESULTS

Immunoblotting

The 671 and C-term hamartin antisera (18, 19) detect single bands at approximately 140 kDa, and the $\alpha\text{p}1$ and $\alpha\text{p}3$ tuberin antisera detect single bands at approximately 190 kDa on Western blots of NT2 cell lysates (Fig. 1, A–C; right-hand panels),

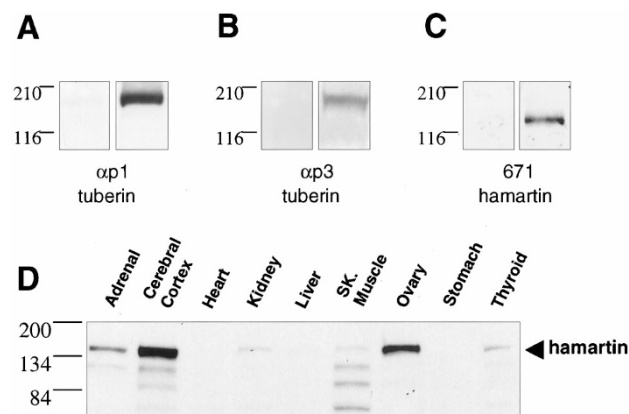


FIGURE 1. Western blots of cultured cells and human tissues with tuberin and hamartin antisera. **A, B** $\alpha\text{p}1$ tuberin (**A**) and $\alpha\text{p}3$ antisera (**B**) detect bands at approximately 190 kDa, the predicted size of tuberin, on blots of NT2 cell lysates (*right-hand panels*). **C** 671 hamartin antiserum detects a single band at approximately 140 kDa, the predicted size of hamartin, in NT2 cell lysate (*right panel*). C-term hamartin antiserum, also used in this study, was described previously (18). **A–C** Preabsorption of the three antisera with their corresponding peptides abolishes staining (*left panels in A–C*). **D** C-term hamartin antisera detects hamartin (*arrowhead*) in adrenal gland, cerebral cortex, kidney, skeletal muscle, ovary, and thyroid gland in addition to lower molecular-weight bands probably representing degradation products in several of these tissues.

lysates of multiple human and rat cell cultures, and human cerebral cortical surgical and some autopsy tissue homogenates (complete data not shown). Immunoreactivities are blocked by peptide absorption (see Fig. 1, A–C; left-hand panels; 18). Western blots performed for this study with the C-term hamartin antibody detected multiple smaller bands in most of the human autopsy tissues collected in addition to the expected band at ~ 140 kDa corresponding to hamartin. An immunoblot with C-term hamartin antibody on human tissues from one patient is shown in Figure 1. Because multiple bands are not detected in surgical tissues and fresh cell lysates, the extra bands are most likely due to tissue autolysis before and during autopsy and represent degradation products of the full-size hamartin protein. Hamartin (~ 140 -kDa band) is detected in homogenates of adrenal gland, brain, kidney, ovary, pituitary, skeletal muscle, and thyroid gland but not in heart, liver, spleen, or stomach.

Immunohistochemistry

The tissue and cell-type distributions of hamartin and tuberin immunoreactivity are summarized in Table 3. Although hamartin and tuberin immunoreactivities varied slightly from case to case, there were no significant differences in the expressions of tuberin or hamartin in comparable tissues from patients of different ages. 671 hamartin antiserum and $\alpha\text{p}3$ tuberin antiserum were used for immunohistochemistry on all 12 cases and the surgical specimens. To verify and strengthen the data from these studies, C-term hamartin antiserum was used on 3 of the 12 autopsy cases and on the surgical specimens. $\alpha\text{p}1$ tuberin antiserum was used on four cases and the surgical specimens. Staining was nearly identical with either hamartin or tuberin antiserum (data not shown); all photographs shown are of tissues stained with either $\alpha\text{p}3$ tuberin or 671 hamartin antisera. Peptide absorption of 671 hamartin and $\alpha\text{p}3$ tuberin antisera were performed on sections of kidney and liver from some cases. Peptide absorption eliminated significant immunoreactivity. Hamartin and tuberin immunoreactivity were localized primarily to the cytoplasm of most cells, but immunohistochemistry with tuberin antisera often also produced strong nuclear and/or perinuclear staining—a result consistent with our immunohistochemical studies of neuroglial cells within TSC-associated cortical tubers (18).

Tuberin and hamartin were found expressed in various human epithelia—including bronchial, gut, glandular epithelia, and renal tubular epithelia—smooth, striated, and cardiac muscle; vascular smooth muscle; and myenteric ganglionic cells. Weak staining throughout lymphatic tissues was produced with our hamartin antisera. Moderate to

TABLE 3. Summary of Tuberin and Hamartin Immunoreactivity in Human Tissues

	Hamartin	Tuberin
Adrenal gland		
Zona glomerulosa	+++	++
Zona fasciculata	+++	++
Zona reticularis	+++	+++
Medulla	++	++
Gut (small/large intestine and colon)		
Epithelium		
Enterocytes	+++	+++
Goblet cells	+++	+++
Smooth muscle	+++	++
Myenteric plexus	+++	+++
Heart (cardiac myocytes)	+++	++
Kidney		
Glomeruli	0	0
Proximal convoluted tubules	++	++
Loop of henle	+ / +++	+ / +++
Distal convoluted tubules	+++	++
Collecting tubules/ducts	+++	++
Liver		
Hepatocytes	++	++
Ductal epithelium	++	++
Lung		
Bronchial epithelium	++	++
Type I & II pneumocytes	0	0
Smooth muscle	+++	++
Lymph node	0 / +	+
Pancreas		
Secretory acini	+ / +++	+ / +++
Islet cells	+++	+ / +++
Ductal epithelium	++	++
Pituitary		
Anterior pituitary chromophils*	+++	++
Anterior pituitary chromophobes	0	0
Posterior pituitary	0	0
Prostate		
Ductal epithelium	+ / +++	++
Smooth muscle	+++	++
Skeletal muscle	+++	++
Spleen		
White pulp	0 / +	0 / +
Red pulp	+	++
Testes		
Seminiferous tubules	++	++
Interstitial cells	++	++
Thymus	0	0
Thyroid	+	+

+++ = prominent staining; ++ = moderate; + = minimal; 0 = staining absent.

* Although cellular staining intensities were similar, fewer cells were stained with anti-tuberin.

prominent tuberin expression was found in macrophages throughout various tissues and monocytes of the splenic red pulp and within lymph node.

Tuberin and hamartin immunoreactivity were prominent in the cytoplasm of enterocytes and goblet cells within the mucosal epithelia of the small and large intestine. In addition, there was moderate hamartin staining of submucosal vascular media. Moderate and prominent immunostaining for tuberin and hamartin, respectively, were noted in the circular and longitudinal smooth-muscle layers. Both proteins are expressed abundantly in clusters of ganglion cells in the submucosa and between the two smooth-muscle layers (Fig. 2, A–B).

Hamartin and tuberin are moderately expressed in hepatocytes. Staining was diffusely cytoplasmic, often appearing more robust in periportal hepatocytes. Sinusoidal lining cells appeared negative for both proteins. Moderate immunoreactivity with both antisera was found in epithelium of bile-collecting ducts (Fig. 3, A–B) and hepatic arteries of the portal tracts.

Tuberin and hamartin are moderately expressed in seminiferous tubules in the testes. Both proteins appear to be expressed in many spermatogonia (Fig. 3, C–D); however, some cells of the tubules were not labeled with either antiserum. Tuberin and hamartin are also moderately expressed in interstitial Leydig cells.

All antisera produced negligible staining within glomeruli of the renal cortex. Hamartin immunoreactivity in the cortex was robust within some cells of the distal convoluted tubules (DCT) and collecting tubules but only moderate in the proximal convoluted tubules (PCT). Conspicuous lack of hamartin immunoreactivity was noted within some cells of the DCT (Fig. 4C). Tuberin immunoreactivity, on the other hand, appeared to be expressed more homogeneously throughout proximal and distal tubules (Fig. 4). This finding contradicts a recent report suggesting that hamartin is more widely expressed than tuberin in renal tubules (19). Tuberin and hamartin are both expressed moderately, with some variability in cellular/tubular staining, within descending and ascending segments of the loops of Henle. Moderate or prominent expression of both tuberin and hamartin were found in cortical and medullary ductal epithelia.

The TSC gene products colocalize in many glandular tissues. Hamartin and tuberin are expressed in all layers of the adrenal cortex—with the highest cortical expressions in the zona reticularis—and within clusters of medullary cells. In the anterior pituitary, many cells with extensive cytoplasm were stained with both antisera (see Fig. 5, A–B). However fewer cells of this type were labeled with tuberin antisera. Smaller cells with scant cytoplasm did not appear stained with any antiserum. Negligible immunoreactivity was noted in cells of the posterior pituitary (pars neurosa).

Tuberin is expressed somewhat homogeneously in secretory acini of the exocrine pancreas, islets of Langerhans, and ductal epithelium. Hamartin immunoreactivity, on the other hand, is slightly less prominent in secretory acini but much more robust in a minority of cells often located in the periphery of the endocrine pancreas (see Fig. 5, C–D). Thyroid follicle lining cells are weakly immunoreactive for both proteins (Fig. 5, E–F).

We detected high expression of tuberin and hamartin in prostate gland. Hamartin was very robust in smooth muscle of the stroma, capsule, and

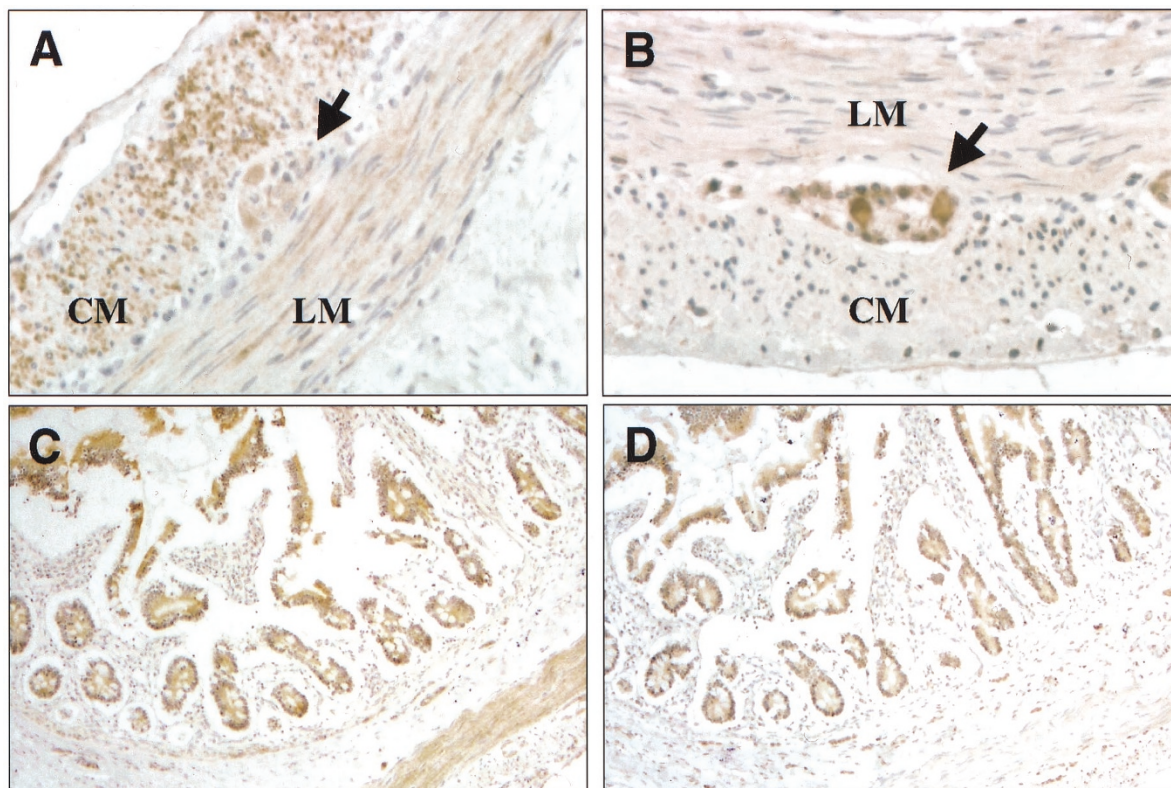


FIGURE 2. Hamartin and tuberin in small intestine of a 4-day-old, full-term infant. **A, B** Hamartin (**A**) immunoreactivity is prominent in the circular (**CM**) and longitudinal smooth-muscle cell layers (**LM**). Note the expression of hamartin in a cluster of parasympathetic ganglion cells (arrow). Tuberin immunoreactivity (**B**) is slightly more intense in the ganglion cells (arrow) but much less robust in the smooth muscle layers (250 \times). **C, D** Both hamartin (**C**) and tuberin (**D**) are abundantly expressed by cells of the mucosal epithelium (90 \times). Staining appears limited to the cytoplasm of enterocytes and goblet cells. Both proteins are expressed in the walls of submucosal blood vessels—although hamartin expression appears more abundant.

vasculature. Hamartin antisera produced weak to moderate variable staining of ductal epithelium. Tuberin expression was often more prominent in the ductal epithelial cells but less prominent in smooth muscle than was hamartin expression.

In lung, type I and II pneumocytes did not appear to express tuberin or hamartin. Some scattered cells—including macrophages—were immunostained with tuberin antisera. Hamartin staining in these scattered cells was weak but often apparent. Tuberin and hamartin were both moderately expressed by bronchial epithelium. As in other organs, prominent hamartin and minimal to moderate tuberin immunoreactivity were found in vascular smooth muscle.

DISCUSSION

We have previously reported that hamartin and tuberin are expressed in similar neuronal populations within the human CNS as early as 20 weeks of gestation (17, 20). This study was performed in order to compare the expression of the TSC gene products tuberin and hamartin in other human tissues at various prenatal and postnatal ages. Immunohistochemistry was performed with two antisera

recognizing distinct peptides of tuberin and two antisera recognizing two distinct peptides of the hamartin protein. Because some of our findings are at variance with data reported previously, we also compared surgical and autopsy specimens from select tissues to control for tissue autolysis. Immunohistochemical patterns of staining in specimens of gut, liver, prostate, pancreas, and kidney removed at surgery were identical to staining patterns in those removed at autopsy. The cellular expression patterns of tuberin and hamartin were also similar in comparable autopsy tissues from our cases ranging in age from 20 weeks of gestation to 8 years of age.

Immunostaining with our antisera suggests that hamartin and tuberin are expressed by the same tissues and cell types, with few variations in the levels of expression. Cellular colocalization of hamartin and tuberin is a logical expectation because the two TSC gene products have been shown to interact (9). However, hamartin is more robustly expressed than tuberin in striated, cardiac, and smooth muscle, distal segments of the nephron, and some cells of the endocrine pancreas.

The most apparent differences in tuberin *versus* hamartin immunoreactivity were those found in

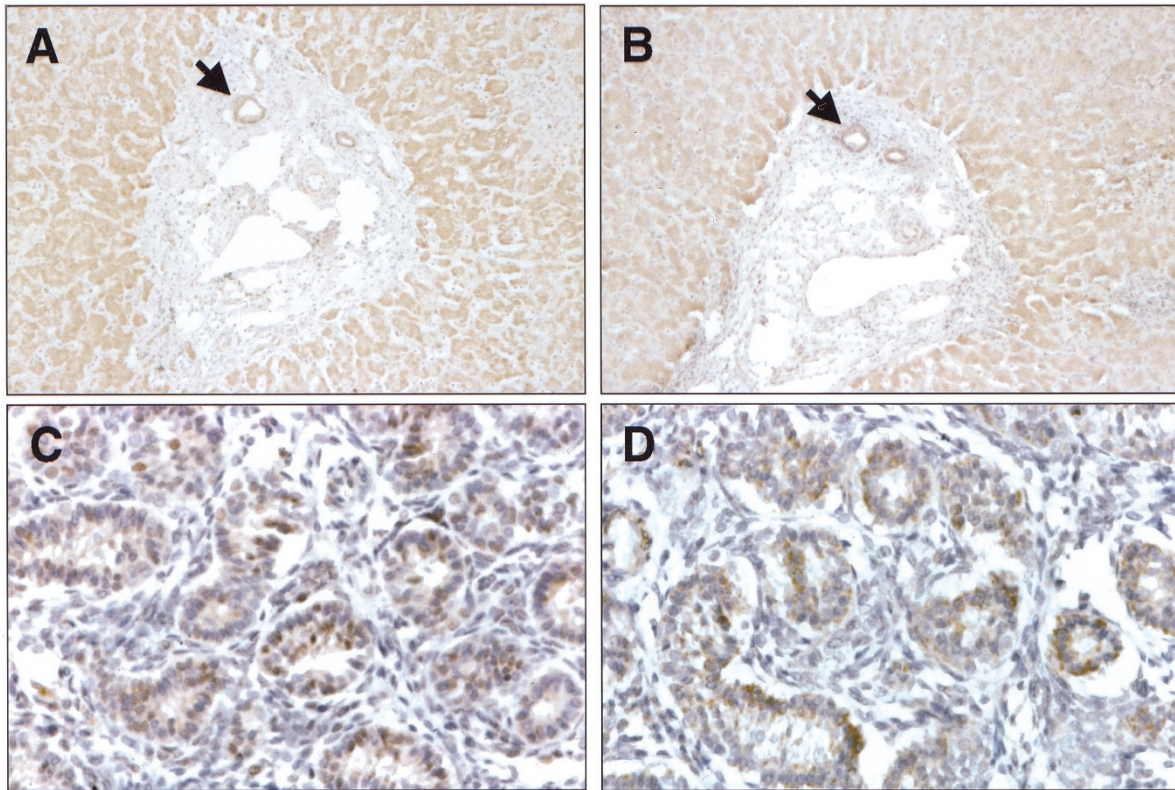


FIGURE 3. Hamartin and tuberin expression in liver and testis of a 3-month-old infant. Sections were stained with anti-hamartin antibody (A, C) and anti-tuberin antibody (B, D). A, B) Serial sections of liver illustrate diffuse cytoplasmic hamartin and tuberin expression in hepatocytes (60 \times). Note moderate expression in bile ductal epithelium (arrows) and arterioles of the portal tract (center field). C, D) Hamartin and tuberin are both moderately expressed in testis (250 \times). Many spermatogonia and interstitial cells are stained with antisera to both TSC gene products.

the kidney and pancreas—with very high hamartin and comparatively low tuberin immunoreactivity in distal convoluted tubules and a small population of islet cells. Because of the similarity of the anti-tuberin and anti-hamartin staining intensity within other tissues in our study and within other cell types of these same tissues, we believe these data support cell-specific differences in TSC gene product expression and do not reflect relative titers of our antisera. The exact significance of these differences in expression is unclear but may suggest that hamartin expression within some cells is regulated by a mechanism that does not regulate tuberin production. Hamartin may perform a unique function in these cells separate from or in addition to its hypothesized functional interaction with tuberin.

In contrast to results from a previous report (17), we have detected tuberin and hamartin in liver, testis, small and large intestine, and prostate (by immunohistochemical staining of autopsy and surgical tissues) and hamartin in ovary (by Western blot). In support of these findings, we find that tuberin and hamartin are detectable, by Western blot, in homogenates of human hepatocarcinoma and prostatic carcinoma cell lines (21). These data are consistent with previous reports of TSC2 transcript and tuberin localization in human and rat

tissues (13, 14, 16). We were unable to detect full-length hamartin on immunoblots of homogenates of liver and heart removed at autopsy, although immunohistochemical staining of paraffin-embedded materials with our antisera suggests that the proteins are present in these organs. This is not surprising, however, given the postmortem interval of the tissues used for immunoblotting and the propensity of these tissues to autolyze rapidly after death. On the basis of our experience with cultured cells and autopsy tissues, hamartin and tuberin are often degraded unless tissue/cell collection and lysis/homogenization procedures are performed quickly and in the presence of several protease inhibitors. We hypothesize that previous inability by others to detect hamartin in some tissues might have been the result of extensive tissue autolysis.

It is interesting that despite the relatively ubiquitous expression patterns of the TSC gene products, the most common TSC-associated hamartomas are limited to relatively few organs—brain, heart, kidney, and skin. As reported by others (17), this is highlighted by the conspicuous rarity of reports detailing endocrine hamartomas associated with TSC, despite expression of the two gene products in normal endocrine tissues. However, reports of endocrine abnormalities associated with TSC have ap-

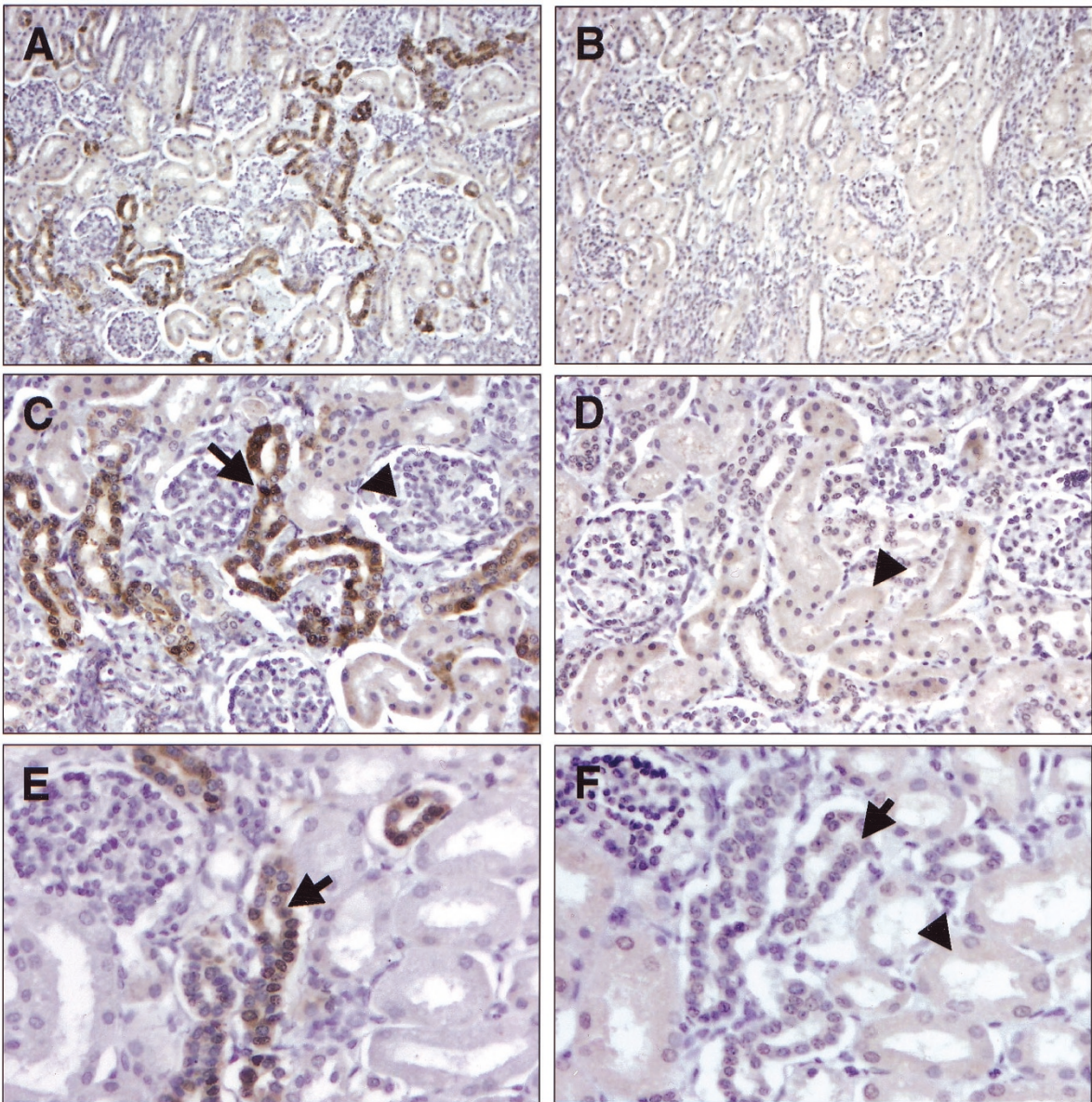


FIGURE 4. Hamartin and tuberlin expression in renal cortex. **A, B** Differences in hamartin (*left*) and tuberlin (*right*) expression are obvious within low-magnification fields (90 \times) of the renal cortex of a 1.5-month old. **C, D** At higher magnification, moderate hamartin (**C**) and tuberlin (**D**) expression are noted in proximal convoluted tubules (arrowheads) with obvious brush borders (170 \times). Hamartin expression, however, is very abundant within some cells of the distal convoluted tubules (*arrow*). **E, F** Similar patterns of expression are noted in similar fields within serial sections of the renal cortex of a 3-month-old (360 \times). Note the minimal expression of tuberlin (**F**) as compared with hamartin (**E**) within distal convoluted tubular epithelium (*arrows*).

peared. Angiomyolipoma of the adrenal gland, thyroid adenoma, pancreatic islet cell adenoma, pituitary adenoma, angiomyolipoma of the testes, hyperparathyroidism, and hypothalamic/pituitary/gonadal abnormalities resulting in precocious puberty have all been reported in patients afflicted with TSC (22). Furthermore, there have been rare reports of tumors and hamartomas associated with TSC in other organs, including liver and intestine (23–27).

Tuberlin has been implicated in numerous cell functions, including cell proliferation and cell cycle control and endocytosis (7, 8, 14). Hamartin inter-

acts with and may function with tuberlin to affect cell proliferation (28), its purported chaperone (29), and has also been shown to interact with an N-terminal peptide sequence common to ERM proteins (10). However, expression profiles of ERM proteins do not entirely correlate with the distribution of hamartin in all human tissues. For instance, ezrin is expressed highly in proximal convoluted tubules and glomerular epithelium of the human renal cortex but only minimally within the distal convoluted tubules (data not shown). Similar localizations of ezrin and moesin have been reported in the rat kidney (30). These cellular distributions are

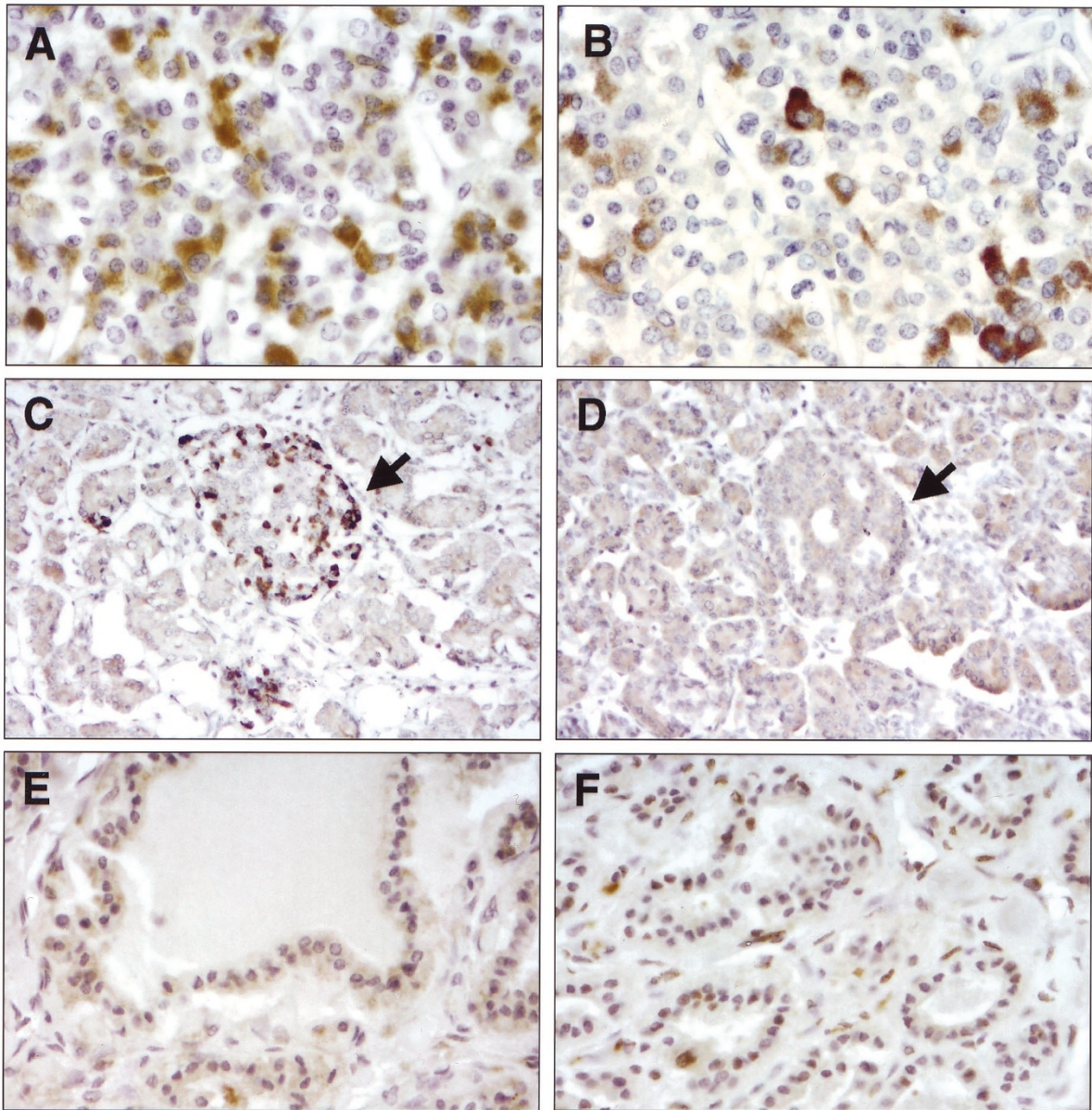


FIGURE 5. Tuberlin and hamartin in endocrine tissues. **A, B** Hamartin (**A**) and tuberlin (**B**) are expressed abundantly by many large chromophilic cells in the anterior pituitary of an 8-year-old (360 \times). More cells in comparable sections appear stained with the hamartin antiserum than with anti-tuberlin. Smaller cells with little cytoplasm do not appear stained with either antiserum. **C, D** Expressions of tuberlin and hamartin are compared in similar fields of serial sections from pancreas (170 \times). Tuberlin expression (**D**) is homogeneously moderate throughout acini and islet cells (*arrow*) from a 3-month-old. Hamartin (**C**) is also modestly expressed throughout the exocrine pancreas but is robustly expressed within a few islet of Langerhans cells (*arrow*). **E, F** Weak to moderate cytoplasmic hamartin immunoreactivity (**E**) is found within follicle-lining cells of the thyroid from a 1-year-old. Tuberlin immunoreactivity (**F**) is often more prominent but primarily localized to the nuclei of rare scattered cells (360 \times).

strikingly different from the localization of hamartin in the kidney. Differences in the expression profiles of hamartin and at least some ERM proteins within human tissues and our data demonstrating differences in tuberlin and hamartin cellular expression all support the hypothesis that there may be several cell-specific functions of the TSC gene products. It is likely that because of these complexities in function, it will remain difficult to correlate entirely TSC gene product expression—or loss of expression—with the common clinicopathologic manifestations of tuberous sclerosis.

In conclusion, tuberlin and hamartin are co-expressed in most human cell types and tissues. Contrary to a previous report, we have found co-expression of hamartin with tuberlin in liver, gut, prostate, and testis and expression of hamartin in ovary. We furthermore describe interesting differences in the cellular expression of the TSC gene product expressions in the kidney and endocrine pancreas, suggesting that hamartin may function in a unique fashion in some cell types of these tissues. Further study of the specific cell types that display differential expression of the TSC gene products

may provide clues pertinent to the individual functions of the two proteins.

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