Technical Advance

The Generation and Characterization of a Cell Line Derived from a Sporadic Renal Angiomyolipoma

Use of Telomerase to Obtain Stable Populations of Cells from Benign Neoplasms

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Angiomyolipomas are benign tumors of the kidney derived from putative perivascular epithelioid cells, that may undergo differentiation into cells with features of melanocytes, smooth muscle, and fat. To gain further insight into angiomyolipomas, we have generated the first human angiomyolipoma cell line by sequential introduction of SV40 large T antigen and human telomerase into human angiomyolipoma cells. These cells show phenotypic characteristics of angiomyolipomas, namely differentiation markers of smooth muscle (smooth muscle actin), adipose tissue (peroxisome proliferator-activator receptor γ , PPAR γ), and melanocytes (microphthalmia, MITF), thus demonstrating that a single cell type can exhibit all of these phenotypes. These cells should serve as a valuable tool to elucidate signal transduction pathways underlying renal angiomyolipomas. (*Am J Pathol* 2001, 159:483–491)

Tuberous sclerosis complex is a relatively common autosomal-dominant disorder affecting 1 in 6000 individuals, characterized by tumors of the kidney, brain, and skin.¹ Brain tumors consist of subependymal nodules and subependymal giant cell astrocytomas, which are low-grade tumors, but may cause ventricular obstruction as a result of continued growth. Kidney tumors include angiomyolipomas,² which begin in childhood, and renal cell carcinoma. Angiomyolipomas may cause renal failure through replacement of the kidney parenchyma with tumor and may lead to life-threatening hemorrhage.³ An elevated incidence of renal cell carcinoma has been observed in tuberous sclerosis complex patients, which may result in metastatic spread and death.⁴ In addition, angiomyolipomas and tumors of perivascular epithelial cells (PEComas) may exhibit malignant behavior, causing death through metastatic growth.⁵ The molecular events responsible for angiomyolipoma development are not fully understood.⁶

Tuberous sclerosis complex is the result of mutations in one of two genes, *Tsc1* (hamartin),⁷ and *Tsc2* (tuberin).^{7,8} The functions of these genes are not completely understood, however, they have been shown to functionally interact with each other.^{9–11} Two mammalian models for tuberous sclerosis complex have been studied. Eker rats have a retroviral insertion in the *Tsc2* gene and develop renal cell carcinomas and splenic angiosarcomas.¹² Mice heterozygous for a targeted mutation in the *Tsc2* gene also develop renal cell carcinomas, but also have a high incidence of hepatic hemangiomas.^{13,14}

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However, neither animal model develops angiomyolipomas. To obtain further insight into signal transduction pathways in angiomyolipoma, we have generated a stable angiomyolipoma cell line by sequentially introducing SV40 large T antigen and human telomerase into tumor cells from a sporadic human angiomyolipoma. The techniques described here are generally applicable to any benign neoplasm. These studies may lead to increased knowledge of signal transduction abnormalities in angiomyolipoma and eventually to medical therapy for angiomyolipoma.

Materials and Methods

Transfection of SV40 into Human Angiomyolipoma Tissue

Viable angiomyolipoma tissue was obtained from a spontaneous renal angiomyolipoma during a total nephrectomy. The patient was a 63-year-old female with no history of tuberous sclerosis complex or physical stigmata characteristic of tuberous sclerosis according to the Gomez criterion. Tumor tissue was dissociated with sterile filtered collagenase type II (Worthington, Lakewood, NJ) in phosphate-buffered saline after manual dissociation through repetitive pipetting with a plastic pipette. Collagenase was neutralized with serum-containing media, and the cells were cultured in type II complete media (50/50 mixture of Dulbecco's modified Eagle medium/Ham F12), supplemented with sodium selenite 5×10^{-8} mol/L, insulin 25 $\mu\text{g/ml}$, hydrocortisone 2×10^{-7} mol/L, transferrin 10 $\mu\text{g/ml}$, T3 (triiodothyronine) 1×10^{-9} mol/L, vasopressin 10 $\mu\text{U/ml}$, cholesterol 1×10^{-8} mol/L, ferrous sulfate 1.6×10^{-6} mol/L, epidermal growth factor 10 ng/ml, and 15% fetal bovine serum (supplied by Elizabeth Henske, Fox Chase Cancer Center, Philadelphia, PA). Two weeks after culture, cells were transfected with 40 μg of the plasmid pMKS-Vori⁻,¹⁵ which encodes the entire genome of SV40 except for a small deletion at the origin of replication, in the presence of 40 μl of lipofectamine (Life Technologies, Inc., Gaithersburg, MD) in 2 ml of complete serumless media (Cellgro, Herndon, VA). Clones were picked from the transfected cells, and one clone was selected for introduction of telomerase based on ultrastructural similarities with authentic angiomyolipomas.

Electron Microscopy

To determine the morphological characteristics of SV40-transfected clones, electron microscopy was performed. Cell pellets were immersed in 4% cacodylate-buffered glutaraldehyde. After fixation, the cells were washed in buffer, fixed in 1% OsO₄ solution, dehydrated in graded ethanols and propylene oxide, and embedded in Embed-812 epoxy resin (Electron Microscopy Sciences, Fort Washington, PA). Thick sections (0.5 $\mu\text{mol/L}$) cut with glass knives were stained with Toluidine Blue. Ultra-thin sections (0.1 $\mu\text{mol/L}$) sections were cut with a diamond knife and mounted on 200-mesh copper grids, stained

with uranyl acetate and lead citrate, and photographed with a Phillips EM201 electron microscope (Phillips, Marburg, Germany). One clone (SV7) that was ultrastructurally most similar to angiomyolipoma was selected for further characterization.

Activation of Telomerase in SV40-Transfected Cells

Because all of the SV 40-transfected cell lines entered senescence after prolonged passage, we decided to overexpress human telomerase into SV7 cells, because these cells ultrastructurally resembled angiomyolipoma tissue. An MMLV-based retroviral vector, LZRS¹⁶ containing the full-length cDNA for human telomerase reverse transcriptase (hTERT; Geron Corp., Menlo Park, CA) was used to activate telomerase as previously described.¹⁷ Briefly, hTERT-LZRS was produced in the Phoenix-packaging cell line after stable transfection and replication-deficient virus was stored at -80°C until ready for use. One day before infection, SV7 cells were plated at 60% confluency in 6-well dishes and on the day of infection, the cells were pre-incubated in Dulbecco's modified Eagle medium containing 10% fetal bovine serum with 5 $\mu\text{g/ml}$ polybrene at 37°C for 10 to 15 minutes. Media was removed and viral stock containing 5 $\mu\text{g/ml}$ of polybrene was added, followed by centrifugation ($300 \times g$) for 1 hour at 32°C . Cells were then incubated for 5 to 6 additional hours at 32°C , followed by continuous passage in original growth media. Activation of telomerase in transduced cells was confirmed by a polymerase chain reaction (PCR)-enzyme-linked immunosorbent assay-based telomere repeat amplification protocol (Roche Molecular Biochemicals, Indianapolis, IN) according to the manufacturer's recommendations. hTERT(+) SV7 cells maintained telomerase activity for at least 40 population doublings and show no activity decrease at the time of this publication. These cells are now known as SV7 tert. SV7 tert cells have been submitted to the American Type Culture Collection for general distribution (ATCC CRL-2461).

Immunohistochemical Analysis of SV7 Tert Cytospins

We performed cytopins of SV7 tert cells to determine whether they demonstrated phenotypic characteristics of angiomyolipoma cells, namely expression of melanocytic and smooth muscle markers. SV7 tert cells were released from tissue culture flasks with trypsin, and trypsin was neutralized by centrifugation of cells through 9 ml of Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum. The resulting cell pellet was fixed in 10% buffered formalin and was embedded in paraffin. Cells were stained with antibodies for muscle-specific actin HHF-35, 1:100, (DAKO Corp., Carpinteria, CA), and microphthalmia transcription factor (MITF) C5+D5, 1:40, (Neomarkers). Negative controls consisted of substitutions of serum for primary antibody.

Molecular Analysis of MITF Expression in SV7 Tert Cells

Reverse transcriptase-PCR was performed using 1 μ g of total RNA isolated from SV7 tert and control melanoma cells (501 mel) with Tth DNA polymerase (Roche Molecular Biochemicals) as suggested by the manufacturer using an MJ Research PCR apparatus (PTC-200). 5' primers were specific for A or M MITF isoform RNA. The 3' primer sequence was common to both RNA species. The M form primer comprises nucleotides 21 to 44 of the M form MITF message with the sequence 5'-CCT TCT CTT TGC CAG TCC ATC TTC-3'. The A form primer comprises nucleotides 170 to 193 of the A form message with the sequence 5'-TGA AGA GCC CAA AAC CTA TTA CGA-3'. The 3' primer comprises the complement of nucleotides 906 to 883 of the A form message with the sequence 5'-GAT CAA TCA AGT TTC CCG AGA CAG-3'. PCR products were visualized after 1% agarose electrophoresis by ethidium bromide staining. For immunoprecipitation and Western blotting, SV7 tert cells or control cells (DTC-1) were lysed and extracts were immunoprecipitated with C5 monoclonal antibody against MITF as described.¹⁸

Analysis of Active Mitogen-Activated Protein Kinase (MAPK) and TSC Protein Expression in SV7 Cells

SV7 cells were analyzed for expression of hamartin, tuberin, and active (phosphorylated) MAPK before introduction of telomerase. To determine whether tuberin, hamartin, and phosphorylated MAPK were appropriately expressed in our cells, proteins were extracted using MAPK lysis buffer (20 mmol/L Tris, pH 7.5, 10 mmol/L EGTA, 40 mmol/L β -glycerophosphate, 1% Nonidet P-40, 2.5 mmol/L magnesium chloride, 2 mmol/L sodium orthovanadate, 1% aprotinin, 1% benzamidine). Equal amounts of protein, as determined using the bicinchoninic acid protocol (Pierce, Rockford, IL) were loaded on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels, using a standard SDS-polyacrylamide gel electrophoresis protocol. After electrophoresis, the gels were transferred to Immobilon-P membranes and probed with a phospho-specific MAPK antibody (New England Biolabs, Beverly, MA), a rabbit polyclonal anti-tuberin antibody (C20; Transduction Laboratory), or a rabbit polyclonal anti-hamartin antibody¹⁹ and developed using horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (ECL; Amersham, Arlington Heights, IL). Equal loading of samples was checked by stripping the blots and re-probing with an antibody to tubulin (clone DM1A; Sigma Chemical Co., St. Louis, MO). C20/A4 cells is a chondrocyte cell line derived by transfection of the pMKSVori- plasmid into primary human chondrocytes, and was used as a control cell line for SV40 expression.²⁰

Co-Immunoprecipitation of Tuberin and Hamartin in SV7 Tert Cells

Cells were lysed in 20 mmol/L HEPES, 150 mmol/L NaCl, 1% Triton X-100, 10% glycerol, 1 mmol/L ethylenediaminetetraacetic acid, 10 mmol/L β -mercaptoethanol, 0.5 mg/ml Pefabloc SC and cleared by centrifugation at 14,000 rpm for 10 minutes. The supernatants were pre-cleared with Protein A/G agarose beads (Santa Cruz Biotechnology, Santa Cruz, CA) and immunoprecipitated with anti-Tsc 1 rabbit antibodies H2 or H4 (1 μ g/ml) and anti Tsc 2 rabbit antibodies: tuberin (C-20) or tuberin (N-19). Prebled serum and purified rabbit IgG (Santa Cruz Biotechnology) served as a control. The immunocomplexes were pulled down with Protein A/G beads and washed three times with Tris-buffered saline buffer. The immunoprecipitates were resolved on SDS/polyacrylamide gel electrophoresis, immunoblotted for TSC1 with HM4 (1:1000) or TSC2 with C-20 or N-19, and detected with ECL (SuperSignal; West Pico, Pierce).

Genetic Analysis of Hamartin and Tuberin in SV7 Tert Cells

DNA was extracted from SV7 tert cells and analyzed according to the procedure of Au and colleagues.²¹

Figure 1. Gross appearance of angiomyolipoma with accompanying kidney removed by total nephrectomy. Note that the tumor, which is white-colored, is nearly as large as the normal kidney.

Primer pairs were developed to flank each exon of hamartin and tuberlin, and amplification was accomplished by PCR. PCR products were electrophoresed at 2 W at 4°C, followed by gel-drying and autoradiography. Bands were examined by single strand conformation polymorphism analysis.

Analysis of akt Phosphorylation and PPAR γ Expression in Angiomyolipoma Cells

Phosphoinositol-3-kinase (PI3K) is a major signal transduction pathway involved in lipogenesis, angiogenesis, and tumorigenesis, and is required for PPAR γ -mediated accentuation of lipogenic differentiation.^{22–24} Thus, we examined SV7 tert cells for expression of phosphorylated akt, a major downstream target of phosphoinositol-3-kinase activation, and expression of PPAR γ . After treatment, the cells were harvested at 5×10^7 /ml in lysis buffer (0.05 mmol/L phosphate buffer, pH 7.4, 5% glycerol, 1 mm ethylenediaminetetraacetic acid) and sonicated briefly to break the cells. Equivalent amounts of protein extraction (10 μ l) were boiled with 10 μ l 2 $\times$ sample buffer (Bio-Rad, Richmond, CA) and loaded on 10% SDS-polyacrylamide gels. After electrophoresis, the gels were transferred to polyvinylidene difluoride membrane at 450 mA for 2 hours in transfer buffer (25 mmol/L Tris, 190 mmol/L glycine, 20% methanol, and 0.05% SDS). The membranes were blocked in block buffer (10 mm Tris, pH 7.5, 0.1% Tween-20, 100 mm NaCl, 5% nonfat dry milk) and probed with a phospho-akt antibody, 1:1000, (New England BioLabs, Beverly, MA) or PPAR γ (E-8) antibody, 1:500, (Santa Cruz Biotechnology). After an extensive wash in Tris plus Tween-20, the membranes were probed with horseradish peroxidase-conjugated secondary antibody and detected by chemiluminescence (Amersham).

Immunohistochemistry for PPAR γ in Angiomyolipoma Tissue

Five- μ m sections of formalin-fixed, paraffin-embedded tissue from the patient's angiomyolipoma were tested for the presence of PPAR γ using anti-PPAR γ antibodies (E-8, Santa Cruz Biotechnology) at a concentration of 1:10 using an avidin-biotin complex technique and steam heat-induced antigen retrieval. An avidin-biotinylated enzyme complex kit (DAKO LSAB2, DAKO Corp.) was used in combination with the automated DAKO Autostainer. Hematoxylin is used as a counterstain. Negative controls were primary antibody replaced by buffer.

Results

Introduction of SV40 into Human Angiomyolipoma Tissue

Primary cells were transfected with a plasmid encoding the entire SV40 genome, except the origin of replication, into cells derived from a spontaneous angiomyolipoma

Figure 2. Electron microscopic appearance of cells derived from the angiomyolipoma after SV40 transfection. **A:** SV3 cells. **B:** SV6 cells. **C:** SV7 cells. Original magnifications, $\times 8155$. Note the absence of secondary lysosomes in SV6 and SV7 cells and fibroblastoid morphology, and presence of secondary lysosomes in SV3 cells (A).

(Figure 1). Approximately 1 month after transfection, foci of cells were noted, and seven foci were expanded. Four of these clones rapidly underwent senescence, and were not studied further. Three clones were examined by electron microscopy.

Electron Microscopy

To further characterize the cells that were expanded from the angiomyolipoma, electron microscopy was performed.

Figure 3. Immunohistochemical analysis of cytopins of SV7 tert cells for microphthalmia (MITF) and smooth muscle actin. **Left:** H&E staining of formalin-fixed SV7 tert cells revealing an epithelioid morphology. **Middle:** SV7 tert cells stained with MITF, a transcription factor seen in melanocytic cells, revealing a characteristic nuclear staining. **Right:** SV7 tert cells stained with smooth muscle actin. The black arrows point to focal cytoplasmic staining for smooth muscle actin.

Two populations of cells were identified (Figure 2). The first population demonstrated a fibroblastic appearance and lack of abundant lysosomes (clone SV3, Figure 2A). The second population demonstrated an epithelioid morphology and abundant secondary lysosomes, characteristic of authentic angiomyolipoma tissue (clones SV6, SV7; Figure 2, B and C).²⁵

Introduction of Telomerase into SV7 Cells

Given that the SV7 cell line exhibited ultrastructural features of authentic angiomyolipoma tissue, we chose

it for further study. To circumvent senescence and obtain a stable population of angiomyolipoma cells, we introduced telomerase into early passage SV7 cells, and obtained cells we named SV7 tert cells.

Immunohistochemical Analysis of SV7 Tert Cytopins

SV7 tert cells exhibited epithelioid features by routine hematoxylin and eosin staining (Figure 3A) and demonstrated nuclear staining with MITF, a melanocytic tran-

MITF expression in AML

Figure 4. MITF expression and isoform analysis in SV7 tert cells. **A:** Total RNA from SV7 tert and control cells was subjected to reverse transcriptase-PCR with primers specific for the MITF A or M isoform mRNA. Products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. The predicted molecular weight of MITF A and M isoform PCR products are indicated. MITF A isoform is the predominant isoform expressed in SV7 tert cells. **B:** Whole cell extracts of SV7 tert or control melanoma cells were immunoprecipitated and Western blotted with C5 anti-MITF monoclonal antibody. MITF A and M isoforms are marked. IgH and IgL refer to the C5 immunoglobulin heavy and light chains.

scription factor previously demonstrated to be expressed in angiomyolipomas (Figure 3B).²⁶ In addition, cells were focally positive for cytoplasmic staining with smooth muscle actin (Figure 3C), consistent with the melanocytic and smooth muscle phenotype observed in angiomyolipomas.

Molecular Analysis of MITF Expression in SV7 Tert Cells

RNA and protein expression of MITF was examined by reverse transcriptase-PCR using primers specific for the M and A isoforms of MITF. Abundant expression of both M and A isoforms were observed in control cells, and abundant A isoform was expressed in SV7 tert cells (Figure 4A). MITF is also expressed on the protein level in SV7 tert cells, with a higher molecular weight A isoform band. This is consistent with the reverse transcriptase-PCR analysis that shows predominant expression of the A isoform of MITF (Figure 4B).

Analysis of SV7 Tert Cells for Function of Hamartin (Tsc1), Tuberin (Tsc2), and MAP Kinase Activity

SV7 tert genomic DNA was analyzed for mutations in *Tsc 1* and *Tsc 2* according to the methods of Au and colleagues.¹⁸ No mutations in either of these genes were observed (data not shown). Demonstration of physical interaction by co-immunoprecipitation of tuberin and hamartin indicates that these genes were functional in SV7 tert cells (data not shown). All cells analyzed demonstrated expression of hamartin and tuberin (Figure 5). These proteins are the same size as native tuberin and hamartin. In addition, SV7, but not control C20/A4 cells, demonstrated activation of MAP kinase signaling through increased expression of phosphorylated (active) MAP kinase (Figure 5).

Analysis of akt Phosphorylation and PPAR γ Expression in SV7 Tert Cells

In addition to MAPK activation, we wished to determine whether the PI3K signal transduction pathway was activated.^{22,27} A primary activity of activation of the phosphoinositol-3-kinase pathway is phosphorylation of akt, a kinase involved in regulation of apoptosis. High levels of phosphorylated akt were observed, and these levels were down-regulated by the PI3K inhibitor LY294002, indicating that phosphorylation of akt in these cells is dependent on PI3K activity (Figure 6A).

PPAR γ is a molecule implicated in lipocytic differentiation, and activators of PPAR γ have been demonstrated to have therapeutic activity in liposarcoma.²⁸ SV7 tert cells demonstrated expression of PPAR γ (Figure 6B), suggesting that this gene may be involved in the lipomatous differentiation of angiomyolipomas. Immunohistochemistry of the spontaneous angiomyolipoma from which SV7 tert cells were derived shows abundant staining of cells lining the large diameter vessels (Figure 7).

Figure 5. Western blot analysis of angiomyolipoma cell lines. Analysis of expression of hamartin, tuberin, and MAP kinase. Equal amounts of total protein (75 μ g) were separated by SDS-polyacrylamide gel electrophoresis and analyzed with antibodies specific for tuberin (C20), hamartin, activated phospho-MAPK, and tubulin. In all cell lines, tuberin and hamartin were expressed. Increased MAPK activation was only observed in SV7 cells and not the C20 control. Equal amounts of total protein were confirmed using the tubulin monoclonal antibody. Ten μ g of protein were electrophoresed through acrylamide gels. C20 represents C20/A4 cells, an SV40 large T-immortalized human chondrocyte cell line, and SV7 represents SV7 cells. Protein extracts were analyzed for tuberin, hamartin, phosphorylated MAP kinase, and tubulin as a control for loading.

In Vivo Tumorigenicity

Five million cells were injected into 2-month-old male nude mice, in the presence or absence of Matrigel (Collaborative Bioscience, Waltham, MA). No tumors were observed after 4 months of observation.

Discussion

Angiomyolipomas are uncommon renal tumors that have smooth muscle, fat, and vascular components. They are found spontaneously in 1 of 1000 humans, and are the most common renal tumor in tuberous sclerosis complex, an autosomal dominant disorder characterized by benign and malignant tumors of the kidney, brain, and skin. Angiomyolipomas are the leading cause of morbidity and mortality in adults with tuberous sclerosis complex;²⁹ however, study of these tumors has been hindered by

Figure 6. Western analysis of phosphorylated akt and PPAR γ in SV7 tert cells. **A:** Expression of phosphorylated akt in the absence (—) or presence (+) of LY294002, a specific inhibitor of phosphoinositol-3-kinase. Each treatment condition was repeated in triplicate and a representative blot is shown. **B:** Expression of PPAR γ in duplicate lanes of SV7 tert cells

lack of angiomyolipoma-derived cell lines or an animal model that develops this tumor type.

The precise cell of origin of angiomyolipomas is not identified, although they are speculated to arise from perivascular epithelioid cells (PEC cells).^{2,6} Angiomyolipoma is a member of a group of neoplasms that co-express melanocytic and smooth muscle markers, including lymphangio-myomatosis of the lung, and clear-cell sugar tumors.^{30,31} They are most commonly observed in tuberous sclerosis complex, but may occur sporadically, especially in the case of renal angiomyolipomas.^{32,33} This study is the first to describe the use of telomerase in obtaining distinct populations from a benign neoplasm, and will facilitate biochemical and genetic analysis of benign neoplasms, which are composed of admixtures of fibroblasts and neoplastic cells.

Angiomyolipomas have previously been shown to express markers of melanocytic differentiation, including the transcription factor MITF.²⁶ MITF has been shown to exist in at least four isoforms, differing in the 5' end. MITF M is melanocytic-specific, whereas MITF A, the isoform expressed in our SV7 tert cells, is highly expressed phys-

Figure 7. Immunohistochemical expression of PPAR γ in tumor tissue from which SV7 tert cells are derived. **A:** Low-power view of the patient's angiomyolipoma stained with PPAR γ , showing diffuse positivity surrounding a large diameter vessel. **B:** A higher powered view, accentuating the nuclear localization of the PPAR γ staining.

ologically in retinal pigment epithelium.³⁴ Both MITF M and A isoforms potentially transactivate melanocytic genes such as tyrosinase, and mice deficient in MITF M are white-colored with dark eyes.³⁵ The ocular pigmentation is because of MITF A activity.³⁶ We thus demonstrate that SV7 tert cells express MITF A mRNA and protein, similarly to MITF A expression in retinal pigment epithelium and osteoclasts.^{37,38}

Telomerase has been extremely useful in obtaining stable populations of primary cells.^{39–41} Mesenchymal cells rapidly immortalize on overexpression of telomerase, whereas epithelial cells may require additional events, such as inactivation of p16.^{42,43} Remarkably, *in vitro* generation of human tumor cells requires introduction of other oncogenes, such as SV40 large T antigen and oncogenic H-ras, in addition to telomerase.⁴⁴ The relative genetic stability of cells transduced with telomerase may allow *in vivo* reconstitution of organs with cells expanded from a patient, thus reducing the need for allogeneic organ transplantation.^{45–47}

In a survey of renal neoplasms, telomerase activity was detected in renal cell carcinoma, but not in angiomyolipoma or oncocytoma.⁴⁸ A second case report of a hepatic angiomyolipoma detected no evidence of telomerase expression.⁴⁹ Telomerase expression may be a late event in the rare transformation of benign angiomyolipomas to malignant PEComas. The lack of expression of

telomerase in benign neoplasms such as angiomyolipoma may explain in part the difficulty in obtaining stable populations of cells from angiomyolipomas.

The availability of angiomyolipoma cell lines will be useful in elucidating biochemical and genetic aberrations that underlie this usually benign but clinically morbid tumor. In addition, we demonstrate the general utility of telomerase in obtaining and expanding populations of cells from benign neoplasms that should facilitate genetic and biochemical analysis of other benign tumors. Telomerase has been used to obtain stable populations of normal cells with preservation of phenotypic characteristics.^{42,50,51} We extend these findings to benign neoplasms. Currently, analysis of benign neoplasms is hindered by admixture of tumor stromal cells that may confound biochemical and genetic analysis.⁵² Knowledge of the signal transduction abnormalities in angiomyolipoma may result in novel medical therapy for these tumors, both in tuberous sclerosis and in spontaneous angiomyolipomas.

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