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REVIEW

Tuberous sclerosis complex: genetic basis and management strategies

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Abstract: Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder that results from mutations in the *TSC1* or *TSC2* genes. TSC is a multisystem hamartoma syndrome with manifestations in the brain, heart, lungs, kidney, skin, and eyes. Neurologically, TSC patients may exhibit severe epilepsy, cognitive disabilities, and autism spectrum disorders. Many TSC patients also present with renal angiomyolipomas, polycystic kidney disease, skin lesions, and lymphangiomyomatosis. TSC1 and TSC2 proteins form a heterodimeric complex that serves to inhibit mammalian target of rapamycin (mTOR) signaling pathway through Ras homolog enriched in brain (Rheb). TSC1 and TSC2 receive activating or inhibitory signals from multiple inputs including growth factors, insulin signaling, energy and amino acid levels, and proinflammatory pathways, which are then integrated to regulate the activity of the mTOR pathway. mTOR signaling plays a critical role in regulating cell growth, transcription, translation, and autophagy. Animal models have shed light on certain features of TSC, but failed to recapitulate the disease completely and currently further research is under way to better understand this devastating disorder. Clinical trials with mTOR inhibitors have shown promising results for some features of TSC, but further research needs to be conducted to establish full indications for therapeutic treatment.

Keywords: tuberous sclerosis complex, TSC, TSC1, TSC2

Tuberous sclerosis complex

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder with an incidence of one in 6000–10,000 live births.[1,2] Currently an estimated one million individuals are affected worldwide, involving all racial and ethnic groups. TSC is characterized by hamartomas, or benign tumor-like growths, in multiple organs including brain, lungs, heart, kidney, skin, and eyes.[3–5] TSC exhibits both variable penetrance, with individuals from the same family showing differential severity of specific features, and pleiotropy, in which individuals sharing similar genotypes have disparate clinical manifestations. TSC is diagnosed according to a group of major and minor diagnostic criteria (see Table 1), which were revised at an NIH-sponsored consensus conference in 2004.[5] Genetic testing is valuable in confirming an early diagnosis but is not currently considered requisite for clinical diagnosis.

Clinical diagnostic features

Neurological manifestations

Neurological disorders are among the most common causes of morbidity in TSC patients. Individuals with TSC exhibit epilepsy, cognitive disabilities, and autism

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Table 1 TSC clinical diagnostic criteria[5]

	Major features	Minor features
Brain	Cortical tuber	Cerebral white-matter radial migration lines
	SEN	
	SEGA	
Lungs	LAM	
Heart	Cardiac rhabdomyoma	
Kidney	Renal AML	Multiple renal cysts
Skin	Facial angiofibromas	"Confetti" skin lesions
	Ungual or periungual fibroma	
	Hypomelanotic macules (three or more)	
	Shagreen patch	
Eyes	Retinal nodular hamartomas	Retinal achromatic patch
Other		Multiple pits in dental enamel
		Hamartomatous rectal polyps
		Bone cysts
		Gingival fibromas
Definitive TSC		
Two major features		
One major plus two minor features		
Probable TSC		
One major plus one minor feature		
Possible TSC		
One major feature		
Two or more minor features		

Abbreviations: AML, angiomyolipoma; LAM, lymphangiomyomatosis; SEGA, subependymal giant cell astrocytoma; SEN, subependymal nodule.

spectrum disorders.[3] Nearly 90% of TSC patients develop epilepsy throughout their lifetime, which is often progressive and intractable to medications. TSC is also the most common genetic cause of infantile spasms, a devastating epilepsy syndrome that affects 30%–40% newborn infants. Approximately 50%–60% of TSC patients exhibit behavioral abnormalities, cognitive disabilities, and autism spectrum disorders. With increasing numbers of cases that are diagnosed prenatally or in early infancy, prior to seizure onset, questions regarding possible prophylactic anticonvulsant therapy to prevent development of epilepsy have emerged.[6]

TSC brain lesions include developmental brain malformations known as cortical tubers, subependymal nodules (SENs), and subependymal giant cell astrocytomas (SEGAs). Cortical tubers are present in 80% of TSC patients and are characterized histopathologically by loss of normal six-layered structure of the cerebral cortex. Tubers are composed of abnormal dysmorphic neurons, 'giant' cells (GCs), and proliferative astrocytes, which have abnormal cellular morphology, cytomegaly, aberrant axonal projections, and dendritic arbors.[7] Fetal tubers have been identified as early as 20 weeks gestation[8] and it is currently believed that *TSC1* and *TSC2* mutations alter the normal development of neural precursors between 7 and 20 weeks.[9] A recent study utilizing magnetic resonance imaging (MRI) has described distinct cortical tuber types based on signal intensity of subcortical white matter.[10] Tubers Type A were isointense on volumetric T1 images and had subtle hyperintensity on T2-weighted and fluid-attenuated inversion recovery (FLAIR); Type B were hypointense on volumetric T1, but hyperintense on T2-weighted and FLAIR; and Type C were hypointense on volumetric T1 images, hyperintense on T2-weighted, and heterogeneous on FLAIR.[10] Furthermore, this study compared and correlated TSC manifestations in patients with different tuber types: Type A patients had a milder phenotype, whereas patients with predominantly Type C tubers had other MRI abnormalities in addition to tubers, such as SEGAs, and a higher probability of having autism spectrum disorders, a history of infantile spasms, and a higher frequency of epileptic seizures, compared to patients with Type A and Type B tubers.[10]

In the few reported neuropathological analyses of postmortem TSC brains, disruption of normal brain architecture distinct from tubers included small structural abnormalities including heterotopias, subcortical nodules, radial migration lines, areas of hypomyelination, and small cortical dysplasias.[7,11] These lesions differ from tubers in that they are smaller, GCs are an infrequent finding, cortical lamination is only mildly altered, and they do not exhibit calcification. Recent MRI analyses in TSC patients have confirmed subtle structural abnormalities outside of tubers in the cortex and within subcortical structures such as the thalamus and basal ganglia[12,13] and suggest that these non-tuber brain lesions, in addition to tubers, may contribute to autism and cognitive disability in TSC.

SENs are nodular lesions typically less than 1 cm in size and are located on the surfaces of the lateral and third ventricles. SENs are present in about 80% of TSC patients and are believed to be asymptomatic, ie, not related to cognitive deficits or epilepsy. Typically, SENs are covered by a thin layer of ependyma, can exhibit extensive vascularization, and extend into the periventricular white matter and the basal ganglia. These lesions develop early, in fetal life, and often degenerate or calcify later in life.

It is widely believed that SENs transition to form SEGAs, although the molecular mechanisms governing transformation from SEN to SEGA are unknown. SEGAs generally appear within the first 20 years of life. SEGAs generally exceed 1 cm in diameter but can grow greater than 10 cm in size. SEGAs extend into the lateral ventricle and often obstruct the flow of cerebrospinal fluid through the lateral ventricle and foramen

of Monro, causing hydrocephalus, focal neurological deficits, and death. Thus in a select group of TSC patients, SEGAs require surgical removal. Overall, SEGAs are relatively rare and represent only about 1%–2% of pediatric brain tumors. While SEGAs can occur as sporadic tumors, most of these likely represent somatic mosaic TSC cases, ie, TSC gene mutation occurring within a restricted population of cells within a limited number of organ systems.

Dermatological features

Skin lesions are detected at all ages in more than 90% of patients and serve as important clinical diagnostic features in both children and adults with TSC. For example, hypopigmented macules ('ash leaf spots'), are a major diagnostic feature of TSC generally detected in infancy or early childhood. Hypopigmented macules are generally a few millimeters to centimeters in size and can be found anywhere on the face, limbs, or trunk. The Shagreen patch is an area of roughened skin over the lumbosacral or flank region usually a few centimeters in diameter, identified with increasing incidence after the age of 5 years. Ungual fibromas are fleshy growths near or beneath the nail that typically appear after puberty and may develop at any time in later adulthood. Facial angiofibromas (formerly referred to as adenoma sebaceum) may be detected at any age but are generally more common in late childhood or adolescence. They appear around the malar region of the face and the chin but can also be found within the nose and external ear.

Renal lesions

Over 80% of TSC patients have renal manifestations, including angiomyolipomas (AMLs) and polycystic kidney disease. Renal AMLs are benign tumors comprised of abnormal blood vessels, smooth muscle cells, and adipocytes. While AMLs can occur sporadically in TSC patients, multiple AMLs are typically found in both kidneys (bilateral). It is estimated that AMLs can be detected in 55%–75% of adult TSC patients. One study of 25 boys and 35 girls reported that 75% percent of children with TSC had renal AMLs by age 10.5 years.[14] AMLs are detected by ultrasound, computed tomography, or MRI of the abdomen. Because AMLs contain abnormal vasculature (which often contains aneurysms), spontaneous and potentially life-threatening hemorrhage is an important complication. Current treatment of AMLs includes embolization or systemic treatment with sirolimus.[15,16] Rarely, surgery is indicated. In addition to AMLs, TSC patients may develop cysts, polycystic kidney disease, and renal cell carcinomas (RCC, see "TSC and cancer predisposition" section). Epithelial cysts, which can be multiple and are generally asymptomatic, may also be associated with hypertension and renal failure. Two to three percent of TSC patients carry a contiguous germline deletion, affecting both *TSC2* and *PKD1* genes on 16p13, resulting in polycystic kidney disease renal insufficiency.

Pulmonary manifestations

Lymphangiomyomatosis (also called lymphangioleiomyomatosis or LAM) affects women almost exclusively, and is characterized by widespread pulmonary proliferation of abnormal smooth muscle cells and cystic changes within the lung parenchyma (see review by Yu et al, 2010).[17] LAM often presents clinically with dyspnea or pneumothorax during early adulthood. While LAM can occur as a sporadic disorder, the incidence of radiographic evidence of LAM among women with TSC is 26%–39%. Many women with radiographic evidence of LAM are clinically asymptomatic.

Recent studies have focused on understanding whether LAM results as a consequence of metastasis of benign tumors from other parts of the body. Approximately 60% of women who have sporadic LAM also present with renal AMLs. Genetic analyses and fluorescent in situ hybridization studies of recurrent LAM following lung transplantation provide support for benign tumor metastasis, since cells with the same gene mutation were found in the transplanted allograft.[18]

Cardiac manifestations

Cardiac rhabdomyomas develop in approximately 50% of the TSC patients and may result in ventricular obstruction, arrhythmias, or congestive heart failure. However, in most TSC patients rhabdomyomas regress spontaneously with time and many disappear by the first year of life. As a rule, new rhabdomyomas do not appear in later life. In TSC patients with cardiac rhabodmyomas, medications are prescribed to treat arrhythmias and congestive heart failure, and some undergo surgery to relieve ventricular obstruction.

TSC and cancer predisposition

TSC is not classically defined as a cancer predisposition syndrome and few epidemiological studies have accurately assessed the cumulative risk of developing, for example, RCC, in TSC. RCC occurs in TSC in 1%–3% of patients and likely presents at an earlier age than the general population. Conversely, mutations in *TSC1* or *TSC2* have been reported in several sporadic cancers such as transitional cell cancer of the bladder,[19–22] urothelial carcinoma,[23,24] and neuroendocrine tumors.[25] These tumors are not part of the diagnostic criteria

for TSC, and thus their relation to the pathogenesis of TSC is unknown.

Genetics

TSC results from mutations in *TSC1* (9q34) or *TSC2* (16p13.3) gene.[26,27] *TSC1* is an 8.6 kb transcript, with a total genomic extent of 55 kb, consisting of 23 exons, and encoding an 1164 amino acid, 130 kD protein TSC1 (hamartin).[27] *TSC2* is a 5.5 kb transcript, with a total genomic extent of 40 kb, consisting of 41 exons, and encoding an 1807 amino acid, 180 kD protein TSC2 (tuberin). Approximately 20% of affected TSC individuals have an inherited *TSC1* or *TSC2* mutation, while in 80%, TSC results from a sporadic mutation. Over 1000 unique TSC1 and TSC2 allelic variants have been reported due to nonsense, missense, insertion, and deletion mutations, involving nearly all exons of *TSC1* and *TSC2*.[28–34] A study examining the differences between patients with *TSC1* versus *TSC2* mutations, found that individuals with sporadic *TSC1* mutations had an age range, average age, and median age that was similar to patients with sporadic *TSC2* mutations.[34] However, TSC patients with a sporadic *TSC1* gene mutation had on average milder disease manifestations, in particular neurological manifestations, than patients with *TSC2* mutations of similar age. Germline and somatic mutations were more common in *TSC2* gene than in *TSC1*,[34] and a subset of patients did not have any identifiable mutation in *TSC1* or *TSC2* gene. In another study, in a cohort of 362 patients, 276 had a definite clinical diagnosis of TSC and had a mutation detection rate of 85%.[31] However, approximately 15% had no identifiable mutation in either *TSC1* or *TSC2*, which could have been due to large deletions, somatic mosaicism, or an unidentified locus. When examining the spectrum of TSC gene mutations, mutations in *TSC2* were 3.4 times more common than in *TSC1*.[31] In this study, *TSC1* mutations and familial *TSC2* mutations were associated with less severe phenotypes than sporadic *TSC2* mutations.[31] In a more recent study in 325 patients, mutations in either *TSC1* or *TSC2* genes were identified in 72% of de novo and 77% of familial cases, but 29% of patients had no mutation identified.[35] The current estimate is that mutations in *TSC1* or *TSC2* genes have been identified in 70%–90% of TSC patients, however 10%–15% have no identified mutation.[36]

Aside from broad associations, there are few genotype–phenotype correlations. Prenatal molecular diagnosis using amniocentesis and chorionic villus sampling has been shown to be accurate in 48/50 fetal cases at risk with TSC due to family history or fetal detection of cardiac rhabdomyoma on ultrasound, showing promise for early TSC diagnosis.[37]

While loss of heterozygosity has been reported for hamartomas in almost all TSC lesions,[38–45] there is no consensus on the mechanism of cortical tuber formation in the brain. A recent report implementing single cell sequencing of *TSC1* and *TSC2* in phosphorylated ribosomal protein S6 (P-S6) immunolabeled GCs showed that tubers contain both germline and somatic mutations suggesting a mechanism of biallelic gene inactivation.[46] In an animal model of TSC that is discussed in a subsequent section of this review, a second 'hit' was focally induced on a heterozygous background for a *Tsc1* mutation and resulted in cellular abnormalities reminiscent of tubers,[47] providing support for biallelic gene inactivation in tuber formation. However another group reported that a second mutational 'hit' in *TSC1*, *TSC2*, or *KRAS* is a rare event in tubers.[48] Thus further investigation will need to be conducted to determine the molecular mechanism of cortical tuber formation in TSC.

Role of TSC1 and TSC2 proteins in cellular function

TSC1 and TSC2 proteins have been shown to regulate multiple cellular processes in both mTOR-dependent and mTOR-independent mechanisms. TSC1 and TSC2 proteins form a heterodimeric complex that serves as an upstream regulator of the mTOR pathway. TSC2 acts as a GTPase-activating protein towards Ras homolog enriched in brain (Rheb), which results in inhibition of mammalian target of rapamycin (mTOR) signaling (Figure 1).[49] TSC1 protein stabilizes TSC2 by binding to it and prevents its ubiquitination.[50,51] mTOR is an evolutionarily conserved serine/threonine kinase that integrates signals from various inputs including growth factors, nutrients, energy, and stresses, to regulate multiple cellular processes such as growth, transcription, translation, and autophagy (Figure 1).[50,52,53] mTOR is found in two functionally distinct complexes: mTOR complex 1 (mTORC1), which is comprised of mTOR, raptor (regulatory associated protein of mTOR) and PRAS40, and mTORC2, which is made up of mTOR, rictor (rapamycin insensitive component of mTOR), mSin1, and Protor1/2.[54]

mTORC1 regulates ribosome biogenesis, transcription, translation, and autophagy[52] via phosphorylation of several downstream effector proteins including S6K1, S6, and 4E-BP1.[55] Loss of function mutations in *TSC1* or *TSC2* lead to aberrant activation of mTORC1 signaling, resulting in increased phosphorylation of S6K1, S6, and 4E-BP1.[55] Notch signaling is an important regulator of progenitor cell self-renewal, proliferation, differentiation, and survival.[56] Reduction in Notch1/Jagged1 signaling in vivo decreases the number of

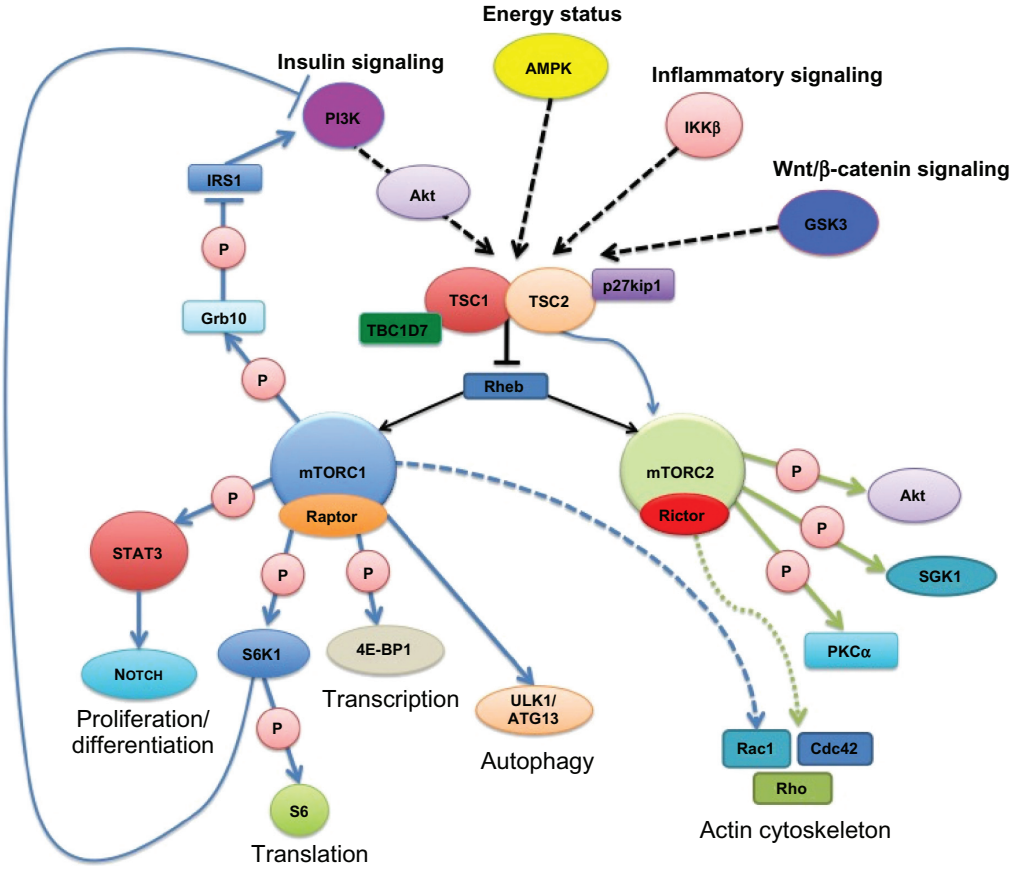

Figure 1 TSC1-TSC2 signaling pathway. TSC1 and TSC2 proteins form a heterodimeric complex that serves as an inhibitor of mammalian target of rapamycin (mTOR) signaling pathway through GTPase Rheb. mTOR forms two distinct complexes with other proteins, among them raptor, specific to mTOR complex 1 (mTORC1) and rictor, specific to mTORC2, to regulate different aspects of cellular function, including transcription, translation, proliferation, differentiation, and autophagy. TSC1 and TSC2 integrate signals from various inputs upstream, among them insulin signaling, energy status, inflammatory, and Wnt/b-catenin signaling, and regulate mTOR pathway activity accordingly.

Abbreviations: 4E-BP1, Eukaryotic translation initiation factor 4E-binding protein 1; Akt, also known as protein kinase B (PKB); AMPK, 5'-adenosine monophosphate-activated protein kinase; Cdc42, Cell division control protein 42 homolog; a Rho family GTPase; Grb10, growth factor receptor-bound protein 10; GSK3, Glycogen synthase kinase 3; IKKβ, IkappaB kinase beta; IRS1, insulin receptor substrate 1; mTORC1, mTOR (mammalian target of rapamycin) complex 1; mTORC2, mTOR (mammalian target of rapamycin) complex 2; Notch, Notch receptor; Notch signaling pathway; p27kip1, cyclin-dependent kinase inhibitor p27; p27 kinase inhibitory protein 1; PI3K, Phophotidylinositol-3-kinase; PKCα, Protein kinase C alpha; Rac1, Ras-related C3 botulinum toxin substrate 1; a Rho family GTPase; Raptor, Regulatory-associated protein of mTOR; Rictor, Rapamycin-insensitive component of mTOR; Rho, Rho family GTPase; S6, Ribosomal protein S6; S6K1, Ribosomal protein S6 kinase 1 (p70S6K1); SGK1, Serum and glucocorticoid-inducible kinase 1; STAT3, Signal transducer and activator of transcription 3; TBC1D7, TBC1 domain family, member 7; TSC1, Tuberous Sclerosis protein 1 (Hamartin); TSC2, Tuberous Sclerosis protein 2 (Tuberin); ULK1/ATG13, Unc-51-like kinase 1; autophagy-related 13 homolog.

proliferating cells in postnatal subventricular zone.[57] A recent study showed that mTOR regulates differentiation through STAT3-p63-Jagged1-Notch pathway in TSC fibroblast, LAM, and mouse kidney tumor cells.[58] A recent phosphoproteome analysis suggested that mTORC1 may actually modulate phosphorylation of several hundred proteins thus positioning TSC1:TSC2:mTOR as a pivotal signaling node in many types of undifferentiated and differentiated cells.[59,60] Rapamycin is a macrolide antibiotic that is a highly specific mTORC1 inhibitor, functioning through FKBP12.[61,62]

Much less is known about mTORC2 signaling and function, but its effectors include Akt, serum and glucocorticoid-inducible kinase 1 (SGK1), and PKCα.[63,64] mTORC2 has been shown to regulate actin cytoskeletal organization and hyperactivated mTORC2 signaling results in altered cell motility in endothelial cells and glioma cell lines,[65,66] although the mechanisms are unknown. mTORC2 is relatively insensitive to immediate direct inhibition by rapamycin;[53] however, long-term treatment in mammalian cells can prevent de novo mTORC2 assembly.[67] Small molecule inhibitor Torin1 has been shown to inhibit both mTORC1 and mTORC2 signaling.[68] While TSC1-TSC2 complex serves an inhibitory role on mTORC1 signaling, some studies have reported opposite effects on mTORC2, and showed that TSC1-TSC2 is required for its proper activation. A study in renal AMLs and *Tsc2*$^{+/-}$ mouse kidney tumors has reported that while mTORC1 biomarkers are increased in TSC tissues, mTORC2 effectors are attenuated.[69–71] However, further investigation

needs to be conducted to understand mTORC2 signaling dysregulation in TSC.

Tsc1 protein has been found to interact with the ezrin-radixin-moesin family of actin-binding proteins.[72] Another binding partner of TSC1, known as TBC1 domain family, member 7 (TBC1D7), may play pivotal roles in regulating the GAP activity effects exerted on Rheb. TSC1 stabilizes TBC1D7, and overexpression of TSC1 results in increased levels of TBC1D7 and its knockdown in reduced levels of TBC1D7.[73] Knockdown of TBC1D7 using siRNA resulted in inhibition of cell growth in lung cancer cells, whereas transplantation of COS-7 cells overexpressing TBC1D7 into BALB/cAJcl-*nu*/*nu* mice resulted in tumor development.[73] Thus future investigation needs to be conducted into the role of TBC1D7 in regulation of mTOR pathway and TSC pathogenesis. Tsc2 has been shown to directly bind to p27kip1 and regulates its cellular localization and stability by preventing degradation by SCF-type E3 ubiquitin ligase complex.[74–79] p27kip1 is a cyclin-dependent kinase inhibitor of G_1 cell cycle progression and regulates proliferation. Akt phosphorylates Tsc2 on Ser939 and Thr1462, and thus controls its nuclear and cytoplasmic localization.[74] In G_0 arrested cells, Akt is downregulated and majority of Tsc2 is localized to the nucleus, however, when the cells re-enter cell cycle, Akt is upregulated, Tsc2 is phosphorylated, and in turn is primarily found in the cytoplasm.[74] Interestingly, S6K1 is found in both the nucleus and cytoplasm, but when it is phosphorylated (Thr389) by mTORC1, it becomes predominantly localized in the nucleus.[80] This shows that phosphorylation events in the mTOR signaling pathway affect protein cellular localization.

Tsc1 knockout (KO) or *Tsc2* shRNA knockdown in hippocampal pyramidal neurons results in enlarged cell somas and altered dendritic spine morphology that were dependent on cofilin Ser3 phosphorylation.[81] These findings implicated regulation of actin cytoskeletal dynamics as the underlying molecular mechanism for aberrant neuronal structural changes following loss of either *Tsc1* or *Tsc2*.[81] A recent study utilizing scratch-induced polarization "wound healing" assay in *Tsc2*$^{-/-}$ fibroblasts demonstrated that Tsc2 has a critical role in cell spreading, polarity, and migration by regulating Cdc42 and Rac1 GTPase activation.[82] Rapamycin treatment rescued the cell polarization defect in *Tsc2*$^{-/-}$ fibroblasts and increased the activation of Cdc42 and Rac1, thus demonstrating mTORC1-dependence.[82] mTORC2 has been shown to regulate the actin cytoskeleton and its deactivation by rictor shRNA knockdown leads to stress fiber formation and delocalized paxillin (an adapter protein present at the junction between actin cytoskeleton and plasma membrane) staining, which is phenotypically similar to *Tsc2*$^{-/-}$ HeLa cells.[83] Further studies will need to be conducted in order to determine whether regulation of cell migration by Tsc1-Tsc2 is through mTORC1 or mTORC2 signaling pathways.

Animal models of tuberous sclerosis complex

Animal models have provided invaluable insight into TSC disease pathogenesis and cellular pathophysiology. Early studies in *Drosophila* showed that inactivating mutations in *dTsc1* and *dTsc2* causes indistinguishable phenotypes with deregulation of various processes, including increased cell size and enhanced cell proliferation.[84–87] These findings led to the identification of the link between *dTsc1*, *dTsc2*, and insulin growth factor signaling, and ultimately to the role of mTOR in TSC. Since then, the Eker rat, which has a spontaneous mutation in the *Tsc2* gene (an insertion that results in production of abnormal larger protein), has been described as an autosomal dominant hereditary TSC animal model with predispositions to renal adenoma and carcinoma.[88,89] Eker rats develop kidney cystadenoma lesions by 4 months, and pituitary adenomas, uterine leiomyomas, and leiomyosarcomas, and splenic hemangiosarcomas between 14 months and 2 years.[90,91] Loss of heterozygosity is seen in the majority of these tumors and established *Tsc2* as a tumor suppressor gene.

More recently, transgenic strategies in mice have resulted in the generation of several different *Tsc1* and *Tsc2* KO models (see Table 2 for details). *Tsc1* or *Tsc2* KO (*Tsc1*$^{-/-}$, *Tsc2*$^{-/-}$) results in embryonic lethality. Specifically, *Tsc1*$^{-/-}$ mice die at E9.5–13.5 and have developmental delay, liver hypoplasia, neural tube closure defects, and poor abdominal organ development.[92–94] *Tsc2*$^{-/-}$ mice die earlier than *Tsc1*$^{-/-}$ (between E9.5–12.5) and also have developmental delay, neural tube closure defects, exencephaly, liver hypoplasia, poor development of abdominal organs, and thickened myocardia.[95–98]

Heterozygote *Tsc1*$^{+/-}$ and *Tsc2*$^{+/-}$ mice develop bilateral renal cystadenomas, liver hemangiomas, lung adenomas and extremity angiosarcomas by 15 months of age and lesion development is milder in *Tsc1*$^{+/-}$ mice compared to *Tsc2*$^{+/-}$ mice[92–98] (see Table 2; for a detailed review see Kwiatkowski, 2010[99]). Rapamycin and other related mTORC1 inhibitors have been shown to be effective in blocking tumor development in *Tsc1*$^{+/-}$ and *Tsc2*$^{+/-}$ mouse models, similar to the results seen in the Eker rat model.[100–102] Furthermore, rapamycin treatment resulted in a decrease in

Table 2 TSC mouse models

Gene	Knockout condition	Phenotype
Tsc1	Neo insertion and deletion of exons 6–8[92]	**KO:** embryonic lethal (E10.5–11.5) due to neural tube closure defects, exencephaly, abnormal morphology of myocardial cells, developmental delay, liver hypoplasia **HET:** kidney cysts and cystadenomas, liver hemangiomas, tail hemangioma, uterine leiomyoma/leiomyosarcoma
	Deletion of exons 17–18[93]	**KO:** embryonic lethal (E9–13.5) due to liver hypoplasia; developmental delay of approximately 1 embryonic day compared to littermates, poor development of abdominal organs, enlarged heart which was shifted inferiorly, pericardial effusions, circulatory failure due to anemia **HET:** bilateral kidney cystadenomas, liver hemangiomas (females: higher % affected, higher average grade; compared to males), forepaw angiosarcoma; premature death (higher in females than in males)
	Neo cassette insertion and deletion of exons 6–8[94,110]	**KO:** embryonic lethal (E10.5–12.5), developmental delay, exencephaly, abnormal vacuolation of myocardial cells **HET:** kidney lesions (cysts, cystadenomas, solid carcinomas), metastatic renal cell carcinomas, liver hemangiomas, premature death; severity of phenotype was dependent on genetic background; impaired hippocampal-dependent learning and impaired social behavior
	Conditional *GFAP-Cre* (target: astrocytes), exons 17–18[105]	**cKO:** megalencephaly, epilepsy, astrocytic proliferation, aberrant hippocampal neuronal organization, premature death
	Conditional *Synapsin1-Cre* (target: neurons), exons 17–18[103]	**cKO:** spontaneous seizures (10%), neuropathological abnormalities (ectopic, enlarged, aberrant neurons), reduced myelination, delayed developmental beginning
	Conditional *Synapsin1-Cre* (target: neurons), exons 17–18[104]	**cKO:** bicuculline-induced epileptiform discharges, hyperexcitability, tonic spasms leading to death
	Conditional *Nestin-Cre* (target: neural progenitors), exons 17–18[114]	**cKO:** structural abnormalities resembling features of SENs and SEGAs in the lateral ventricle
	Conditional *Nestin-Cre* (target: neural progenitors), exons 17–18[115]	**cKO:** enlarged brains, early lethality due to hypoglycemia, poor mother-pup interaction
	Conditional *Emx1-Cre* (target: neural progenitors of the forebrain), exons 17–18[106]	**cKO:** enlarged brain size, enlarged cells, decreased myelination, premature death
	Focal deletion of exons 17–18 in brain on background of *Tsc1*$^{fl/mut}$ [47]	**Focal brain KO:** ectopic cytomegalic and multinucleated neurons, lower seizure threshold
Tsc2	Eker rat; spontaneous insertion mutation[88–91,116–118] (d)	Predisposition to kidney cystadenomas and renal cell carcinomas, pituitary adenoma, uterine leiomyomas, leiomyosarcomas, splenic hemangiosarcomas, some brain lesions
	Neo cassette insertion into exon 2[95] (d)	**KO:** embryonic lethal (E9.5–12.5) due to liver hypoplasia; exencephaly, developmental delay of 1–2 embryonic days compared to littermate, poor development of abdominal organs, heart shifted inferiorly, pericardial effusions, circulatory failure due to anemia **HET:** kidney tumors (renal cysts and adenomas), renal cell carcinoma, liver hemangiomas, lung adenomas, and foot, tail, lip angiosarcomas; deficits in hippocampal-dependent learning
	Neo cassette insertion into exon 2 and deletion of exons 2–5[96] (d)	**KO:** embryonic lethal (E9–12.5) due to neural tube closure defects, exencephaly, abnormal thickened myocardia **HET:** multiple renal cell carcinomas, liver hemangiomas
	Neo cassette insertion into exon 1, deletion of exons 2–4[97] (d)	**KO:** embryonic lethal (E9.5–17); neural tube closure defects, developmental delay **HET:** kidney cysts and tumors
	Deletion of exon 3 (hypomorphic allele, *del3*)[98]	**KO:** embryonic lethal (E9.5–13.5; longer survival compared to previous *Tsc2* KO models[95,96]), developmental delay, liver hypoplasia, poor/deficient hematopoiesis, hemorrhage in multiple sites (heart, liver)

(*Continued*)

Table 2 (*Continued*)

Gene	Knockout condition	Phenotype
		HET: kidney cysts and cystadenomas; phenotype less severe than that of previous *Tsc2* KO models[95,96]
	Conditional Insulin2-Cre (target: pancreatic β-cells), exons 3–4[119]	**cKO:** hypoglycemia and hyperinsulinemia (age 4–28 weeks); hyperglycemia and hypoinsulinemia (after age 40 weeks)
	Conditional *hGFAP-Cre* (target: radial glial progenitor cells), exons 2–4 [97,107]	**cKO:** megalencephaly, cellular cytomegaly, cortical and hippocampal lamination defects, astrocytosis, abnormal myelination, premature death
	Conditional *GFAP-Cre* (target: astrocytes), exons 2–4[108]	**cKO:** megalencephaly, hippocampal neuronal disorganization, astrocytic proliferation, premature death (phenotype more severe than *Tsc1 GFAP-Cre* cKO[120])
	Dominant negative transgene (delta RG)[109,121]	fibrovascular collagenoma in dermis, subpial external granule cells in cerebellum; deficits in social behavior and rotarod learning

Abbreviations: cKO, conditional knockout; HET, heterozygous; KO, knockout; SEGA, subependymal giant cell astrocytoma; SEN, subependymal nodule; TSC, tuberous sclerosis complex.

the size of renal and pituitary tumors and improved survival, however, evidence of drug resistance was reported in a small percentage of lesions after long-term therapy.[101]

Several conditional knockout (cKO) TSC mouse models were generated subsequently. Neuronal *Tsc1* cKO in mice (*Tsc1*$^{fl/fl}$; *Synapsin1-Cre*) results in spontaneous seizures in 10% of mice, ectopic, enlarged, and aberrant neurons, reduced myelination,[103] hyperexcitability and tonic spasms leading to premature death.[104] Mice with *Tsc1* cKO in astrocytes (*Tsc1*$^{fl/fl}$; *GFAP-Cre*) have megalencephaly, epilepsy, increased astrocytic proliferation, aberrant hippocampal organization, and die prematurely.[105] *Tsc1* cKO in the forebrain (*Tsc1*$^{fl/fl}$; *Emx1-Cre*) results in enlarged brain size and cytomegalic cells within the cerebral cortex, and the mice die by postnatal day 25.[106] Recently, a new model of focal *Tsc1* KO in a subpopulation of progenitor cells on a heterozygous *Tsc1* background was described and the mice show aberrant lamination of the cerebral cortex, cytomegalic multinucleated neurons in the intermediate zone (similar to subcortical white matter in humans), and lower seizure threshold, providing support for biallelic gene inactivation in the brain.[47]

Radial glia-specific *Tsc2* cKO mice (*Tsc2*$^{fl/ko}$; *hGFAP-Cre*) have many of the TSC features, including megalencephaly, cellular cytomegaly, and cortical lamination defects.[107] *Tsc2* cKO in astrocytes (*Tsc2*$^{fl/fl}$; *GFAP-Cre*) results in a more severe epilepsy phenotype than *Tsc1* cKO (*Tsc1*$^{fl/fl}$; *GFAP-Cre*), with an earlier onset and higher seizure frequency that were correlated with higher mTORC1 activation.[108] These findings support the theory that mutations in *Tsc2* gene result in a more severe phenotype than mutations in *Tsc1*. Another *Tsc2* animal model that expresses a dominant negative *Tsc2* transgene shows mild but statistically significant impairments in social behavior and rotarod motor learning, recapitulating some of the behavioral abnormalities observed in TSC patients.[109] The dominant negative Tsc2 is able to bind Tsc1, but the mutation affects its GAP domain and rabaptin-5 binding motif.[109]

Tsc1$^{+/-}$ neurons with a single deleted copy of *Tsc1* exhibit morphological changes characteristic of *Tsc1*- and *Tsc2*-deficient neurons, suggesting that haploinsufficiency rather than a complete lack of either *Tsc* gene could contribute to certain aspects of TSC neuropathogenesis.[81] While heterozygote *Tsc1*$^{+/-}$ and *Tsc2*$^{+/-}$ mice do not exhibit gross brain abnormalities, they have cognitive and social behavior deficits and impaired hippocampus-dependent learning.[109–111] *Tsc2*$^{+/-}$ mice have also been shown to have aberrant retinogeniculate projections with EphA receptor-dependent axon guidance in the visual system.[112] This suggests that while there may be no gross apparent brain architectural changes due to *Tsc1* or *Tsc2* haploinsufficiency, there could be alterations in network circuitry.

Rapamycin treatment has been shown to be effective in brain abnormalities in TSC mouse models. Rapamycin treatment started prior to the onset of seizures prevented the development of epilepsy in *Tsc1* cKO mice (*Tsc1*$^{fl/fl}$; *GFAP-Cre*) and improved survival.[113] If the treatment was stopped, however, the neurologic phenotype subsequently developed with a delay of several weeks, including the histopathologic abnormalities and epilepsy.[113] When treatment was started after epilepsy onset, rapamycin reduced the seizure frequency, thus supporting mTOR's role in early and late epileptogenesis, but its effects were not as robust as when rapamycin was begun early.[113] Rapamycin treatment in heterozygous *Tsc2*$^{+/-}$ mice reversed the learning abnormalities, thus demonstrating its potential in treatment of cognitive deficits in TSC.[111]

Recently, in a model of *Tsc1* cKO in the postnatal subventricular zone using a tamoxifen-inducible *Nestin-CreER*^{T2} mouse line, tamoxifen was administered at postnatal day 7 or 1 month, resulting in enlarged brains at 3 and 6–7 months, but had no body weight differences.[114] Furthermore, *Tsc1-Nestin* cKO mice had hydrocephalus, an enlarged hippocampus, and small nodular structures and tumors were present near the interventricular foramen, reminiscent of SENs and SEGAs seen in TSC patients.[114] Most cells in these tumors had enlarged somas and stained positive for mature neuronal markers MAP2 and NeuN or astrocytic markers S100β and GFAP, but were low in Ki67 and did not exhibit multinucleation.[114] Another model of *Tsc1 Nestin-Cre* cKO exhibited normal body weight and organ development, but an enlarged head, and the mice died within 24 hours after birth with lethality being most likely due to malnutrition, hypoglycemia, and hypothermia.[115] The mutant brains grossly showed normal brain architecture, but the cerebral cortex was especially enlarged.[115] Single rapamycin dose (1 mg/kg) was administered subcutaneously to the pregnant dam between embryonic days E15–17, and significantly increased the survival of the mutant mice up to postnatal day 20.[115] This study strengthens the potential of early rapamycin therapy in TSC.

In summary, TSC animal models have taught us a lot about TSC pathophysiology in certain organ systems. The existing TSC animal models have, however, failed to recapitulate all lesions seen in TSC human patients. For example, cortical tubers and LAM lesions have not been completely modeled in animal models. Further investigation and better TSC animal models will be pivotal in the understanding of the disease mechanisms leading to TSC pathogenesis.

Clinical management strategies for TSC

Up until 2007, treatment of TSC was largely symptomatic and not specific for the cell signaling pathways activated in TSC. Thus, anti-epileptic drugs and epilepsy surgery remain the mainstays of epilepsy therapy. Embolization or surgery is used for renal lesions, and oxygen supplementation can provide symptomatic relief for LAM. However, an initial clinical trial assessed the efficacy of sirolimus in reducing the volume of renal AMLs and showed improving pulmonary function tests in LAM.[122] A pivotal finding of this trial was that while AMLs did in fact show diminished volume after 12 months of rapamycin treatment, in the ensuing 12 months during which rapamycin was discontinued, there was re-growth of AMLs in many patients.[122] Phase II clinical trials with sirolimus showed that patients treated for 52 weeks had regression of kidney AMLs, SEGAs, and liver AMLs.[123] Most recently, the mTOR inhibitor everolimus showed efficacy in reducing SEGA volume after 6 months of treatment.[124] Furthermore, there was modest reduction in seizure frequency in nine out of sixteen TSC patients with seizures, but seizure frequency did not change in six individuals, and worsened in one patient.[124] These studies provided clear evidence that modulation of the mTOR pathway in TSC could benefit some patients and thus opened the conceptual door for syndrome specific therapy in TSC. Everolimus is the first mTOR inhibitor that has been FDA approved for treatment of SEGAs associated with TSC.[125] Recently, there has also been a case report of regression of cardiac rhabdomyoma in a TSC patient 13 months after everolimus treatment.[126] While cardiac rhabdomyomas have been shown to regress naturally, the time course in this specific patient who was diagnosed in utero and had no significant changes for the next 5 years, suggests that everolimus treatment might have played a role in the regression and near resolution of the rhabdomyoma.[126] These results support the role of mTOR involvement in TSC pathogenesis and demonstrate the potential of mTOR inhibitors as therapeutic treatments. However, a clear and overarching clinical challenge associated with the use of mTOR inhibitors is the need for continued therapy to prevent recurrence of lesion growth. The modest or non-effect of everolimus on epilepsy necessitates further investigation into the role of mTOR in epileptogenesis in TSC.

Conclusion

In summary, human and animal studies have provided insight into many features of TSC pathogenesis, but certain challenges remain. TSC is a multisystem disorder, with distinct organ-specific manifestations. The Eker rat and *Tsc1* and *Tsc2* cKO mouse models have been instrumental in defining certain aspects of TSC pathogenesis, but have failed to fully recapitulate all features seen in TSC patients. Specifically, animal models of TSC have provided valuable insight into mTOR signaling as a target pathway and provided a pivotal platform to test mTOR inhibitors for renal and neurological features. These preclinical studies demonstrated that mTOR inhibition with rapamycin resulted in better outcomes when begun early, suggesting that mTOR inhibitors be considered as preventative therapies. These studies have further guided clinical trials for the use of mTOR inhibitors in TSC patients and have been shown

to be effective for renal and liver AMLs, LAM, SEGAs, cardiac rhabdomyomas, and possibly, epilepsy. However, the partial efficacy and symptom recrudescence following cessation of treatment merits further investigation into the TSC pathogenesis. Since mTOR signaling has multiple feedback loops, it would be important to examine the downstream targets of mTOR and whether its inhibition results in activation of compensatory mechanisms that could lead to a more severe phenotype. Future genetic studies and new animal models that recapitulate TSC features more closely will provide invaluable insights into TSC pathogenesis in different organ systems.

Disclosure

The authors declare no conflicts of interest in this work.

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