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TSC1/TSC2 Signaling in the CNS

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Abstract

Over the past several years, the study of a hereditary tumor syndrome, tuberous sclerosis complex (TSC), has shed light on the regulation of cellular proliferation and growth. TSC is an autosomal dominant disorder that is due to inactivating mutations in *TSC1* or *TSC2* and characterized by benign tumors (hamartomas) involving multiple organ systems. The TSC1/2 complex has been found to play a crucial role in an evolutionarily-conserved signaling pathway that regulates cell growth: the mTORC1 pathway. This pathway promotes anabolic processes and inhibits catabolic processes in response to extracellular and intracellular factors. Findings in cancer biology have reinforced the critical role for TSC1/2 in cell growth and proliferation. In contrast to cancer cells, in the CNS, the TSC1/2 complex not only regulates cell growth/proliferation, but also orchestrates an intricate and finely tuned system that has distinctive roles under different conditions, depending on cell type, stage of development, and subcellular localization. Overall, TSC1/2 signaling in the CNS, via its multi-faceted roles, contributes to proper neural connectivity. Here, we will review the TSC signaling in the CNS.

Keywords

mTOR; autism; translation

Introduction

TSC is a multisystem disorder, in which 90–95% of the affected individuals have CNS symptoms or signs. Neurologically, TSC can manifest with intellectual disability, behavioral abnormalities, autism spectrum disorders (ASD), and seizures [1]. Epilepsy occurs in 80% to 90% of all patients, often with medically refractory seizures. Close to 45% of patients have mild-to-profound intellectual disabilities and ASD occurs in up to 50% of patients [1,2]. TSC can be diagnosed in the pre- or perinatal period [3], and many neuropathological features such as cortical tubers and histological abnormalities are present by the second trimester *in utero* indicating that neurological manifestations of the disease develop during the embryonic period [4,5]. Clinical signs can be variable with some individuals within a family having minimal symptoms while others carrying the same mutation being severely affected.

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The neuropathological findings in the brain usually take the form of (1) subependymal nodules, (2) subependymal giant cell astrocytomas (SEGA) and (3) cortical tubers [6]. Subependymal nodules are lesions found along the wall of the lateral ventricles in the brain. In 5–10% of cases, these benign lesions can grow into SEGAs that block the circulation of cerebrospinal fluid resulting in hydrocephalus. Tubers are made up of a collection of abnormally large neurons and glia and are most commonly found in the cerebral cortex. It has been proposed that the presence of cortical tubers contribute to the severity of the disorder, but studies have presented conflicting findings on which aspects of the tubers are most critical indicators. More recent studies indicate that the tuber volume is a better reflection of the severity of cognitive impairment than tuber number alone, and also that the location of the tubers (frontal/occipital/temporal/cerebellar) has differential associations with comorbid neuropsychiatric disorders [7–9]. Sophisticated analysis of the neuronal function of TSC1/2 genes *in vitro* and in animal models has revolutionized our understanding of the disease mechanisms and potential treatment options.

TSC1 and TSC2 protein complex

TSC1 (on chromosome 9) and *TSC2* (on chromosome 16) are tumor suppressor genes that integrate extrinsic and intrinsic signals of the cellular energy status and growth. Proteins encoded by *TSC1* and TSC2 genes, also known as hamartin and tuberin respectively, bind to each other to form a GTPase activating protein (GAP) complex that plays a critical role in the regulation of protein synthesis, controlling cell growth and size [10]. Both proteins are required for the proper function of the complex, and thus a mutation in either gene is sufficient to cause the clinical disease. TSC1 is required to stabilize TSC2 and prevent its degradation. On the other hand, the functional GAP domain resides in TSC2, making each protein obligatory for each other's functional role. In fact, studies in *Drosophila* have shown that the *Tsc1; Tsc2* double mutants phenocopy either single mutants and that overexpression of both proteins is required to render a gain of function phenotype [11,12]. Nonetheless, patients with *TSC2* mutations have a worse overall prognosis than those with *TSC1* mutations [13], and the conditional *Tsc2* knockout mouse model has a more severe phenotype than the conditional *Tsc1* knockout in the same conditional genetic background [14]. These differences could be due to the fact that the two proteins have additional independent functions. Another possibility is that, although both TSC1 and TSC2 are subject to ubiquitin-mediated degradation if not bound to each other, some enzymatic activity of TSC2 remains and is able to carry out some of its function before its degradation. TSC1, which has no such catalytic domain, would be ineffective in suppressing mTOR activity on its own [15]. Investigations into the protein interactors of TSC1/2 have begun in non-neuronal cell lines [16–18], but the question of which proteins interact with the TSC1/2 complex in CNS cells - at different times during development and at different subcellular locations - has not yet been explored.

The TSC1/2 complex can be regulated post-translationally by several major signaling pathways in cells: PI3K-Akt, ERK and AMPK (Figure 1). The best-characterized function of the TSC1/2 complex is as a downstream target of the phosphatidylinositol 3-kinase (PI3K) pathway that becomes activated upon the binding of growth factors (e.g. IGF or BDNF). Activated PI3K leads to recruitment of PDK1 and the serine/threonine protein kinase Akt, and subsequent phosphorylation/activation of Akt by PDK1. Activated Akt negatively regulates TSC by directly phosphorylating TSC2 on five consensus sites on human TSC2 [19–22]. A second kinase that can phosphorylate and inhibit TSC2 is the extracellular signaling-regulated kinase (ERK) [23]. ERK phosphorylation of TSC2 appears to be particularly important for EphA-receptor mediated regulation of TSC2 [24]. Both active Akt and ERK levels are found to be high in TSC-related cortical tubers and SEGAs, and the inhibition of TSC2 by these kinases has been proposed to represent a

posttranslational mechanism that may further amplify the loss of the first allele of the TSC gene [23,25]. In addition, AMP-activated protein kinase (AMPK) can phosphorylate TSC2 on a different set of residues than Akt and ERK and potentially increase the ability of TSC1/2 to inhibit the mTORC1 activity, thereby protecting cells from excessive energy use during low energy states [26,27]. TSC1 is also negatively regulated by IKK-beta, which physically interacts with and phophorylates TSC1 at its Ser487 and Ser511 residues in response to inflammatory pathway activation [28]. Because TSC1:TSC2 functions as a dimer, regulation of either protein most likely affects its overall activity level. However, relatively little is known about the post-translational modifications affecting the TSC1 protein and the hierarchy of the regulatory modification on the TSC1/2 complex, particularly those involving AMPK and IKK. Furthermore, none of these posttranslational modifications appear to affect the GAP activity of TSC2 per se, but they somehow affect the ability of the TSC1/2 complex to act as a Rheb-GAP in cells. Whether this effect is due to changes in subcellular localization or other cellular mechanisms is not yet clear.

Downstream of TSC: mTORC1 and 2

When active, TSC2 inhibits Ras family GTPase Rheb by stimulating the conversion of Rheb-GTP to Rheb-GDP. Downstream targets of Rheb include the serine-threonine kinase mammalian target of rapamycin (mTOR), a central regulator of protein synthesis. mTOR kinase exists in two distinct functional complexes, mTOR Complex 1 and mTOR Complex 2, defined by two groups of binding partners (Figure 1). mTORC1 is comprised of the core essential components Raptor and LST8, while mTORC2 contains Rictor, LST8, and SIN1. mTORC1 is bound strongly and is quickly inhibited by rapamycin, while mTORC2 inhibition requires prolonged rapamycin treatment, which blocks mTORC2 assembly [29]. However, rapamycin does not fully inhibit mTORC1 function, with some downstream targets being more sensitive than others [30]. mTORC1 phosphorylates and activates ribosomal S6 kinases (S6K1 and S6K2) and inhibits the translational regulator 4E-BP1 – both events that positively regulate translation of 5′ capped mRNAs. mTORC1 phosphorylates S6K1 on Thr389, resulting in phosphorylation of its downstream effectors that increase mRNA translation [31]. Activation of S6Ks leads to phosphorylation of ribosomal protein S6, elongation factor 2 kinase (eEF-2K), programmed cell death protein 4 (PDCD4), and eIF4B, all of which result in increased protein synthesis [32–35]. Unphosphorylated 4E-BP1 is bound to eukaryotic initiation factor 4E (eIF4E), inhibiting its association with the eIF-4F cap-binding complex, thereby blocking translation initiation [36]. When phosphorylated by mTORC1, 4E-BP1 dissociates from the eIF4E complex, initiating mRNA translation [37,38]. Thus, without the functional TSC complex, mTORC1 is hyperactive, resulting in constitutively phosphorylated S6 protein, disinhibited protein synthesis, and subsequent cell growth [39,40]. As a central regulator of cell growth, mTORC1 is sensitive to nutrient and redox states of the cells, and more recently has been shown to be specifically responsive to amino acids through a not yet well defined pathway involving the Rag GTPases [41–43]. The presence of amino acids somehow alters the nucleotide-bound state of a heterodimeric Rag complex at lysosomal membranes, and this creates a docking site for mTORC1 [42]. Once at the lysosomal membrane, mTORC1 encounters Rheb, but it is not yet clear how or if Rheb is targeted to the same endomembranes as mTORC1 and Rag proteins. Whether neuronal mTORC1 has specific function at the lysosome has yet to be investigated, and it would be interesting to find out other cellular localization sites of TSC1/2 and mTORC1 and their relevance to the function of this signaling pathway.

Our understanding of mTORC2 is nascent when compared to mTORC1 (especially in the CNS), but it is emerging as a critical component of the PI3K/mTOR pathway. While TSC1/2 negatively regulates mTORC1, it promotes mTORC2 activity in a Rheb-

independent manner that might involve the direct binding of the TSC1/2 complex to components of the mTORC2 complex [44–46] Once active, mTORC2 phosphorylates and activates AKT, leading to phosphorylation of its downstream effectors including TSC2 [47]. There also appears to be crosstalk between mTORC1 and mTORC2, as S6K1 phosphorylates and inhibits Rictor [48]. Interestingly, loss of the TSC1 or TSC2 leads to a unique cellular scenerio in which mTORC1 is activated and mTORC2 is attenuated. An important implication of these findings is that the ideal treatment for loss of TSC1/2 may require not only mTORC1 inhibition but also mTORC2 activation. As most of these initial studies were performed in non-neuronal cells, it has yet to be seen whether the pathway is conserved in the CNS and how the intricate balance between the two mTOR complexes affects neuronal function. Studies in the last few years have begun to shed light on the role of these proteins in several aspects of neural development and function, and it is becoming clear that TSC1/2 protein complex is a master regulator of neuronal connectivity.

Roles of TSC Complex in neuronal development and function

Axon Specification

In the CNS, almost all neurons have a single axon and multiple dendrites. Establishing this unique polarized structure is critical for proper function and directionality of the flow of information within the CNS. Interestingly, TSC pathway components are expressed in neurons in a polarized manner [49–51]. Overexpression of Tsc1 and Tsc2 suppresses axon formation while loss of Tsc1 or Tsc2 function leads to increased axon number [49]. This critical function of TSC1/2 appears to be related to its ability to regulate levels of a number of proteins such as SAD-A, a kinase required for axon formation in the mouse brain. When TSC is non-functional, SAD-A proteins levels are increased in neurons in an mTOR-dependent manner [49]. Other proteins that are regulated by TSC/mTORC1 include CRMP2, Tau1 and Rap1B, all of which can play a role in neuronal polarity [50,51]. There are likely to be other neurite proteins whose expression is regulated by TSC, and the full repertoire of proteins regulated by TSC/mTORC1 in neurites remains to be identified.

Axon Guidance

One of the most critical steps in neural development is the formation of precise neuronal networks. Importantly, there is growing appreciation of the role of protein translation in axons as a crucial substrate of axonal development. Axons have long been thought to lack active translation as rough ER and polyribosomes are hardly detectable in mammalian axons. However, axons severed from their soma can synthesize proteins readily [52–54] and many functional aspects of the axonal growth cone involve local mRNA translation. It is possible that monoribosome or other functional analogs can be used to synthesize proteins in axons [55]. Using either compartmentalized culture systems or direct laser capture of axons, recent studies have revealed that hundreds of mRNAs are present in axons [56,57]. Blockade of local translation not only affects growth cone collapse and turning in culture systems but also impairs normal axon guidance, circuit development and regeneration [24,58–60]. Indeed, Sema3A-induced growth cone collapse, which requires local translation of RhoA, is blocked by rapamycin treatment [61]. Many other guidance cues, including netrin-1, slit1, ephrins, regulate axonal protein synthesis through either ERK- and/or mTOR-dependent pathways [24,62,63].

One of the first pieces of evidence for aberrant axon guidance in TSC deficient neurons came from *Drosophila*. The investigators showed that increases and decreases in TOR signaling via Rheb correlated respectively with changes in synaptic overgrowth and reduction [64]. Additionally, mutant photoreceptor neurons lacking Tsc1 formed disorganized lamina plexus and aberrant projections into the medulla [64]. More detailed

molecular study done using a *Tsc2* heterozygous mouse model has shown that haploinsufficiency of Tsc is sufficient to produce aberrant neuronal projections. Axons of retinal ganglion cells find their synaptic targets in the lateral geniculate nucleus of the thalamus by interacting with a group of repulsive axon guidance molecules called ephrins, which bind to cell surface receptors called Eph receptors. Tsc2 heterozygous axons display abnormal growth cone collapse in response to ephrins [24]. Therefore, abnormal collapse of these structures in Tsc haploinsufficient neurons and subsequent incorrect projections into the thalamus show the intimate interweaving of the TSC pathway with Ephrin/Eph pathway, which may play a similar role in other axon projections.

Synapse formation and function

It has long been recognized that neuronal soma size and dendritic growth positively correlate with innervation and the release of trophic factors. In particular, BDNF is reported to be involved in regulating dendritic complexitiy and soma size [65]. However, the signaling cascades mediating the effects of such trophic factors are not well understood. Recently, several groups have reported that the PI3K/Akt/mTOR pathway regulates soma size, dendritic arborization and spine morphogenesis [66–68]. Activation of PI3K and Akt both increased cell size and dendritic complexity while inhibition of endogenous PI3K and Akt decreased cell size and dendritic branching [66,67]. These effects appear to be mediated through mTOR, as treatment with rapamycin or mTOR RNAi decreased dendritic branching [66,67]. Interestingly, Tsc1 or Tsc2 loss increased spine length and head width and decreased the density of dendritic spines in hippocampal slice cultures [68]. Similar decrease in spine density was observed in Tsc1 null neurons in vivo [69].

In addition to structural changes in dendrites, the mTOR pathway is reported to play a role in postsynaptic AMPA receptor expression [70]. In Tsc1 deficient hippocampal neurons, the AMPA/NMDA receptor current ratio was significantly increased relative to that in controls, suggesting an aberrant relative enhancement of synaptic AMPA receptors [68]. In wild-type neurons, the effect of mTOR activation on spine formation appears to be immediate in induction of synapse associated proteins Arc, synapsin I, PSD95, and GluR1 [71]. In this study, the investigators used antidepressant ketamine to activate mTOR, which led to increased spine density and increased EPSCs in response to 5-HT in prefrontal cortical neurons [71]. Although the exact mechanism of how the NMDA receptor antagonist ketamine regulates mTOR activation is unclear, it requires ERK and/or Akt, suggesting that TSC is possibly involved as well. Finally, staining of cortical tubers from TSC patients has indicated a decrease in GluR2 and NR2A staining in giant cells and dysplastic neuron cell bodies [72]. Whether these changes also reflect a reduction in cell surface GluR2 and NR2A expression and the mechanisms leading to these changes are not yet clear. Together, the pre- and post-synaptic roles that the TSC/mTOR pathway plays strongly indicate that abnormalities in this pathway are likely to result in defects in synapse formation, elimination and plasticity, likely correlating with the neurological and developmental symptoms of TSC disease.

Axon Regeneration

Regeneration potential of CNS axons following injury has been limited at best. CNS axon regeneration research has focused on inhibitory factors that have thwarted successful outgrowth of the axons, but recently the TSC/mTOR pathway has emerged as a critical intrinsic modulator of the axon’s potential to regenerate after injury. PI3K/AKT pathway is one of the major intracellular responses to neurotrophin regulated axon outgrowth and inhibition of this pathway in neurons reduces the axon growth that occurs in response to growth factor stimulation [73,74]. This intrinsic outgrowth signal may become diminished in adult neurons after development has been completed and cannot be reactivated post-injury.

In fact, embryonic neurons display strong mTOR activity that then diminishes in adult neurons, and the remaining mTOR activity in adult neurons is further suppressed by axonal injury through a yet unknown negative regulator [75]. Consequently, upon conditional deletion of *PTEN* or *Tsc1* in retinal ganglion cells (RGCs), with resulting mTOR activation, crushed axons exhibit robust long distance regeneration and increased cell survival [75]. This regenerative potential is not unique to the optic nerve, and PTEN deletion is also able to enhance sprouting and outgrowth of corticospinal neurons following spinal cord injury, ultimately reforming presynaptic structures [76]. Since PTEN deletion facilitates the regenerated axons' ability to grow slightly more robustly than those lacking Tsc1, it is possible that there are other PTEN regulated targets such as GSK-3β that promote other necessary axon growth functions such as microtubule assembly [77]. It was more recently shown that intraocular inflammation induced oncomodulin and elevation in intracellular cAMP levels, in combination with PTEN deletion, are complementary in bolstering the long-distance axon regeneration, showing the necessity for activation of parallel injury response pathways for more extensive regrowth [78]. Nonetheless, the most critical component of the axon regeneration appears to be mTORC1 dependent, probably because of its ability to promote overall protein translation. Whether the regenerated axons find the correct target and form functional synapses has yet to be investigated, but the current knowledge of mTORC1's role in these processes predicts that a more precise and coordinated type of modulation of mTORC1 activity may prove necessary during the regeneration process rather than a complete hyperactivation by PTEN suppression.

Cellular stress

One of the critical homeostatic mechanisms that cells have evolved against intracellular stress is Unfolded Protein Response (UPR). In normal neurons, prolonged chemical induction of ER stress leads to inhibition of the mTOR pathway. Inhibition of PI3K pathway results in increased cleaved caspase-3, reflecting activation of the apoptotic pathway, similar to the recently demonstrated data with Tsc2-deficient cells [79,80]. Tsc loss results in mTOR dependent ER stress response at baseline, and upon treatment with stress-inducing agents such as thapsigargin, the Tsc2-deficient cells exhibit a lowered threshold for induction of UPR-regulated genes and mitochondrial cell death pathways. More importantly, the lack of Tsc activity leads to increased expression of the pro-apoptotic transcription factor CHOP (C/EBP homologous protein), production of reactive oxygen species (ROS), and susceptibility to apoptosis. Immunohistochemical analysis on human TSC brain sections demonstrate similar upregulation in CHOP and heme oxygenase (HO-1), suggesting that heightened ER stress could lead to selective vulnerability of TSC-deficient neurons to extrinsic insults such as seizures, hypoxia, and environmental toxins. Given the reproducibility across cells lines on which these studies were performed (neurons, MEFs and kidney cells), it is likely that other CNS cells such as astrocytes and oligodendrocytes would also be susceptible to the damage.

Another cellular response regulated by mTOR is autophagy. Autophagy is an evolutionarily conserved "self-eating" mechanism responsible for the removal of long-lived proteins and damaged organelles by the lysosome. During autophagy, double-membrane autophagosomes sequester intracellular components and then fuse with lysosomes to form autolysosomes in which cargo is degraded. Under growth stimulating conditions, mTOR signaling is activated, which results in inhibition of autophagy. Under starvation conditions, mTOR is inhibited, leading to induction of autophagy. After prolonged starvation, mTOR is reactivated, which reduces autophagy and results in the formation of tubules and vesicles thereby restoring lysosome numbers in the cell [81]. Both ER stress [82] and oxidative stress [83] appear to induce autophagy through the TSC/mTOR pathway. As most of these studies

were performed in non-neuronal cells, further studies will be needed to investigate whether TSC1/2 plays similar roles in regulating neuronal autophagy.

CNS Mouse models of TSC

Heterozygous models

There are several mouse models of TSC, and although none manifest the full complement of the CNS phenotype in humans—cortical tubers, subependymal nodules or SEGAs—each has provided valuable insight. The first heterozygous mouse models of TSC established that haploinsufficiency of either *Tsc1* or *Tsc2* causes neurocognitive deficits such as impaired hippocampal-dependent learning, social behavior, synaptic plasticity, learning and memory [84,85]. Tsc2 heterozygous mice also exhibit abnormal mother-pup interaction as measured by ultrasonic vocalizations (USV), establishing these mice as potential models of autism [86]. These neuropsychiatric abnormalities are present without obvious concomitant neuropathological alterations, prompting more rigorous investigation of subtle molecular and circuitry level changes. Heterozygous models display no clear anatomic abnormality under pathologic evaluation, although $Tsc2^{+/-}$ neurons do display abnormal axon guidance [24], providing further support for the hypothesis that abnormal neuronal connectivity may underlie the neurological symptoms in TSC disease.

Neuron-specific models of TSC

Neuron-specific knockout of *Tsc1* in postmitotic neurons has been generated using *Cre* recombinase under the *Synapsin-1* promoter (*SynI-Cre*) [87]. $Tsc1^{flox/flox}$*; SynICre* mice are viable perinatally, but develop tremor and hyperactivity beginning the second week of life and die starting approximately 4–6 weeks postnatally. These mice exhibit several neuropathological abnormalities similar to those seen in TSC patients including enlarged dysplastic neurons throughout the cortex, hippocampus, and other subcortical grey matter regions as well as spontaneous seizure episodes. mTORC1 inhibitor treatment has reversed some neuroanatomical abnormalities associated with clinical TSC including reduction in neuron size and improvements in biochemical/signaling profiles, as well as clinical improvements in body weight, clasping behavior, tremor, seizures and kyphosis [39]. Furthermore, when animals are taken off treatment, myelination and other clinical improvements remained intact for at least two more weeks, indicating that regulation of neuronal mTORC1 is critical not only during neurodevelopment but also for long term maintenance of neuronal function.

Deletion of *Tsc2* from radial glial precursors cells using *hGFAP-Cre* transgenic mice results in lamination defects, cortical enlargement, astrogliosis as well as myelination defects [88]. $Tsc2^{flox/flox}$*; hGFAP-Cre* mice exhibited severe compromise in survival and profound seizure episodes, suggesting again that cortical tubers are not necessary for the observed phenotypes.

Astrocyte-specific model of TSC

Another important observation made from $Tsc1^{+/-}$ and $Tsc2^{+/-}$ mice was the increase in the numbers of astrocytes [89]. As homozygous loss of Tsc1 or Tsc2 results in prenatal death, Gutmann and colleagues generated $Tsc1^{flox/flox}$*; GFAP-Cre* mice to specifically inactivate Tsc1 in astrocytes in order to study the impact of the complete loss of Tsc in these cell types [90]. $Tsc1^{flox/flox}$*; GFAP-Cre* mice demonstrated up to a six-fold increase in GFAP-immunoreactive cells and subsequent enlargement of some cortical regions such as the hippocampus accompanied by alterations in neuronal organization [90]. Additionally, these mice developed electroencephalographically confirmed seizures by two months of age, but they failed to mimic additional manifestations of TSC such as cortical tubers or cortical

lamination defects. The neuropathological phenotypes in these mice were mTORC1-dependent and treatable, as rapamycin treatment prevented development of progressive astrogliosis, abnormal neuronal organization, development of epilepsy and premature death in these mice [91]. Epileptogenesis in these mice was attributed to the increases in extracellular glutamate levels due to decreased astrocytic GLT-1 and GLAST glutamate transporters, and treatment with ceftriaxone to increase the transporter expressions in presymptomatic mice decreased excitotoxic neuronal death and severity of epilepsy [92,93]. Interestingly, when the mice were treated after the onset of seizures, ceftriaxone treatment and subsequent increase in glutamate transporter expression failed to have an effect on seizures, alluding to the importance of early treatments to prevent permanent neuropathological changes [93]. Furthermore, there may be other astrocytic dysfunction or even embryologic/perinatal alterations arising from TSC-deficiency that may contribute to the seizures given that even the early postnatal treatments did not prevent the seizures completely. Therefore, the exact changes in the neuropathology and the critical time of intervention to effectively prevent seizures require further investigation.

Role of TSC in Oligodendrocytes

Much of the focus in the field has been on astrocytes and neurons, perhaps because of their direct contributions to epilepsy and tuber formation. Nonetheless, the correlation between the severity of cognitive impairments and degree of hypomyelination has brought to surface that the TSC/mTOR pathway is also critical in development and function of oligodendrocytes. Myelination deficits are commonly observed in the TSC brain, both focally within tubers and more diffusely [94–96]. Focal white matter deficits are frequently in the subcortical region underlying tubers, highlighted by ectopic neurons, loss of axons, giant cells, and large astrocytes. More diffusely, this congenital defect persists throughout adulthood, infiltrating numerous major intrahemispheric tracts bilaterally causing approximately 15% reduction in white matter volume [97]. Furthermore, diffusion tensor imaging studies indicate that TSC patients have occult damage in the normal appearing white matter and that this damage may contribute to neurocognitive disability in these patients [96,98–100]. Despite its significance, it has been unclear whether the defect in myelination is cell-autonomous due to loss of TSC function in oligodendrocytes or indirectly due to TSC-deficient neuronal dysfunction.

OLs proliferate, differentiate, and myelinate in independently controlled events that require specific extrinsic cues for each stage of their development. Of these, neuron-synthesized insulin-like growth factor-1 (IGF-1) has been shown to affect all three aspects of OL development [101,102]. Interaction of IGF-1 with IGF-1R on OLs result in rapid transcription and *de novo* protein synthesis in the OLs through PI3K/Akt, mTOR, and MEK/ERK pathway activation as shown by respective pharmacological inhibition of each pathway component [103]. mTOR activation is required for the terminal differentiation of the oligodendorcyte precursor cells (OPCs) by enabling intrinsic mechanisms to acquire OL-specific gene expression [104]. Interestingly, mTORC1 and mTORC2 appear to have distinct temporal roles in the process; mTORC1 targets P70S6K1 and 4E-BP for phosphorylation at the onset of OPC differentiation while mTORC2 substrate Akt Ser473 phosphorylation was sustained through latter stages of differentiation [104]. Additionally, other recent studies have shown that hyperactivation of the pathway in OL lineage cells either by selective deletion of PTEN (*Olig2-cre*, $Pten^{fl/fl}$) or constitutive overexpression of Akt (*PLP-Akt-DD*) both result in mTOR-dependent hypermyelination [105,106]. PI3K/mTOR pathway appears to regulate myelination by modulating the amount of protein translation; however, this process seems to be limited to the developmental period, as *Olig2-cre*, $Pten^{fl/fl}$ mice did not exhibit greater remyelination following lysolecithin induced demyelination [105].

Despite their lack of complete demonstration of the human TSC phenotype, each animal model generated has given valuable insight into the function of the TSC/mTOR pathway in each cell type. Since in the CNS, each neuronal and glial cell type has different functions, it is likely that TSC1/2 play a number of roles in the development and function of each of these cell types. Conditional and inducible knockout of Tsc1 and Tsc2 in specific cell types and developmental periods is likely to provide important insights into the CNS biology as well as the pathogenesis of TSC disease.

TSC-related diseases

Findings from TSC may also have implications for other conditions in which mTORC1 is hyperactive. Such conditions include genetic diseases such as Neurofibromatosis type 1 (NF1), Fragile X Syndrome (FXS), and PTEN hamartoma syndrome, all of which have been associated with ASDs, behavioral dysregulation, or intellectual disability. In addition, there is evidence of mTORC1 activation in focal cortical dysplasias [107] and gangliogliomas [108].

NF1 occurs due to loss-of-function mutations in the NF1 tumor suppressor gene. The NF1-encoded protein, neurofibromin, functions as a Ras-GTPase activating protein (RasGAP). In both NF1-deficient primary cells and human tumors, both ras and mTOR are hyperactivated. In fact, the activation of mTORC1 is dependent on endogenous ras activity in these cells [109]. Furthermore, mTORC1 activity is essential for NF1-associated tumorigenesis [110]. These data suggest that mTOR inhibitors may represent a viable therapy for NF1-related malignancies.

FXS is the most common form of inherited intellectual disability and a leading genetic cause of autism [111]. Accumulating evidence over the last few years indicates that TSC and FMRP pathways interact and share several common signaling components. However, precisely how they interact remains an open question. On the one hand, FMRP can be phosphorylated by S6K1, an enzyme downstream of TSC [112]. On the other hand, mTORC1 is hyperactive in Fmr1 knockout neurons [113], and FMRP-deficient cells display increased activity of PI3K, an enzyme upstream of TSC proteins [114]. These findings have led to the hypothesis that hyperactive PI3K/mTORC1 signaling is pathogenic in FXS [113,115,116]. These important similarities and differences between the two genetic diseases justify further systematic analysis of these conditions using mouse models.

The pathways regulating TSC function have been also implicated in childhood neurological problems, particularly autism. PTEN mutations have been detected in a subset of patients with autism and macrocephaly [117]. Furthermore, when the PTEN gene is deleted in subsets of differentiated neurons in the cerebral cortex and hippocampus, mutant mice show a profound decrease in social interaction and nesting [118]. At a cellular level, PTEN null axonal processes are more exuberant and project to a broader area compared to axons in wild-type mice. Taken together, these results suggest that PTEN inactivation in differentiated neurons is associated with increased axonal growth, ectopic axonal projections, and autistic-like behavior in mice. Importantly, rapamycin treatment blocks the anatomical, cellular, and behavioral abnormalities in these knock-out mice.

Future directions

Emerging evidence – from abnormal white matter on neuroimaging of TSC patients to deficits in axonal integrity in animal models – supports the hypothesis that TSC1/2 proteins play crucial roles in neuronal connectivity. In past 20 years since the identification of genetic cause of TSC disease, staggering insights into basic cell biology as well as targeted therapies have been made. Based on clinical trials [119], the U.S. Food and Drug

Administration has approved an mTOR inhibitor everolimus in November 2010 for treatment of SEGAs in TSC patients, who are not candidates for surgical resection. At the same time, there is growing evidence that TSC and rheb may have mTORC1-independent functions [120,121]. As we experimentally dissect the TSC/mTOR pathway using more precise genetic tools, the crucial role of this pathway in multiple areas of neural development and function is becoming clear. To accelerate this progress, it will be important to generate animal models that more closely replicate human disease. Major gaps in our knowledge include: (1) the genetic and non-genetic modifiers of TSC disease that account for the remarkable variability of expression within the human population; (2) cell type and subcellular location specific roles of TSC1/2 and their effectors; (3) mTORC1-independent aspects of TSC regulation of neuronal function; (4) the interplay between the different neurological symptoms of TSC disease (epilepsy, autism etc); (5) the relationship between neuronal energetics and the TSC/mTOR pathway. Studies that address such questions will shed light on the interaction between TSC1/2 genes, environment, and neurodevelopment.

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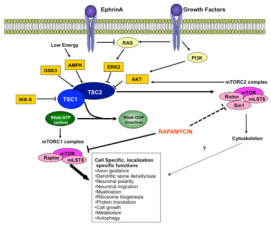

Figure 1.

TSC mediated signaling in the CNS. This cartoon of TSC mediated signaling has been simplified to highlight the demonstrated biologic roles for TSC mediated mTOR signaling in the nervous system.