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The role of mTOR signalling in neurogenesis, insights from tuberous sclerosis complex

Andrew R. Tee^a, Julian R. Sampson^a, Deb K. Pal^b, and Joseph M. Bateman^{c,*}

^aInstitute of Cancer & Genetics, Cardiff University School of Medicine, Institute of Medical Genetics Building, Heath Park, Cardiff CF14 4XN UK

^bDepartment of Basic & Clinical Neurosciences, Institute of Psychiatry, Psychology & Neuroscience, King's College, London SE5 8RX UK

^cWolfson Centre for Age-Related Diseases, King's College London, Guy's Campus, London SE1 1UL UK

Abstract

Understanding the development and function of the nervous system is one of the foremost aims of current biomedical research. The nervous system is generated during a relatively short period of intense neurogenesis that is orchestrated by a number of key molecular signalling pathways. Even subtle defects in the activity of these molecules can have serious repercussions resulting in neurological, neurodevelopmental and neurocognitive problems including epilepsy, intellectual disability and autism. Tuberous sclerosis complex (TSC) is a monogenic disease characterised by these problems and by the formation of benign tumours in multiple organs, including the brain. TSC is caused by mutations in the *TSC1* or *TSC2* gene leading to activation of the mechanistic target of rapamycin (mTOR) signalling pathway. A desire to understand the neurological manifestations of TSC has stimulated research into the role of the mTOR pathway in neurogenesis. In this review we describe TSC neurobiology and how the use of animal model systems has provided insights into the roles of mTOR signalling in neuronal differentiation and migration. Recent progress in this field has identified novel mTOR pathway components regulating neuronal differentiation. The roles of mTOR signalling and aberrant neurogenesis in epilepsy are also discussed. Continuing efforts to understand mTOR neurobiology will help to identify new therapeutic targets for TSC and other neurological diseases.

Keywords

mTOR; Neuronal differentiation; Neuronal migration; Epilepsy; TSC; Neural stem cell

1 Introduction

Tuberous sclerosis complex (TSC) is a monogenic autosomal dominant disease characterised by benign overgrowths in multiple organs [1]. People affected by TSC are commonly beset by neurological disorders including epilepsy, autism and learning disability.

*Corresponding author. joseph_matthew.bateman@kcl.ac.uk (J.M. Bateman).

With the identification of the *TSC1* and *TSC2* genes in the 1990's and the discovery that their encoded proteins, TSC1 and TSC2, functioned upstream of the mechanistic target of rapamycin (mTOR, also known as mammalian target of rapamycin) pathway research into TSC entered the molecular age. Recently, interest in the underlying basis of the neurological manifestations of TSC has led to important breakthroughs in fundamental neurobiology.

In this review we briefly describe the neurological manifestations of TSC and the current understanding of the role that the mTOR pathway has in the neuropathology of TSC. We then discuss how these TSC-related neurological manifestations have stimulated research into the role of mTOR signalling in neural stem cell (NSC) proliferation, neuronal migration and differentiation. We further discuss how activated mTOR signalling contributes to aberrant neurogenesis and has been recently shown to be associated with several congenital cortical malformation syndromes. We do not discuss the role of mTOR signalling in synaptic plasticity, learning and memory, as this has been recently reviewed extensively elsewhere [2–4].

2 Alterations in neurodevelopment and mTOR signalling in TSC

2.1 The neurological manifestations of TSC

The estimated incidence of TSC is around 1 in 6000 newborns [5]. Mutations in the *TSC1* gene, on chromosome 9 (9q34) and the *TSC2* gene, on chromosome 16 (16p13), were identified as being responsible for TSC in the 1990s [6,7]. Until *TSC1* and then *TSC2* were cloned, using patients with TSC as the key resource, the proteins that these genes and their orthologs encode were unknown (in any organism). The discovery in patients with TSC enabled direct experimentation in a wide variety of systems that has illuminated cellular mechanisms across a wide range of biological processes, including neurogenesis. The *TSC1* and *TSC2* protein products, hamartin and tuberlin, interact with each other and together they function as a tumour suppressor. *TSC2* contains an atypical GTPase activating protein (GAP) domain that is essential for the tumour suppressive function of the TSC1/TSC2 protein complex [8]. More than 90% of TSC patients exhibit a wide spectrum of neurological and neuropsychiatric manifestations including epilepsy (including infantile spasms), autism and learning disability, which can begin in infancy; as well as attention deficit hyperactivity disorder, anxiety and depression in adolescence and adulthood [1]. Seizures in TSC are often refractory to treatment with anti-epileptic drugs and early seizure onset (particularly infantile spasms) and difficulty in achieving seizure control are predictive of poor cognitive outcome, although the GABA transaminase inhibitor and mTORC1 inhibitor vigabatrin is frequently beneficial.

TSC patients develop three main types of brain lesions: cortical dysplasia including tubers, subependymal nodules (SENs) and subependymal giant cell astrocytomas (SEGAs). Tubers are present in around 80% of patients and are developmental overgrowths in the cerebral cortex or subcortical white matter. They are characterised by loss of normal cortical lamination, dysplastic neurons, enlarged glia and multinucleate 'giant cells' [9]. Tubers are traditionally thought to be epileptogenic foci, but in some patients seizures still continue after resection of the apparently epileptogenic tuber [10]. SENs, which are thought to be asymptomatic, lie along the lateral and third ventricles and contain enlarged neurons, glia

and giant cells [11]. In 10–15% of patients one or more SENs grow into SEGAs, which express both neuronal and astrocytic markers [12]. Although they are classified as benign tumours, SEGAs can block the flow of cerebrospinal fluid at the foramen of Munro causing obstructive hydrocephalus and death [13].

Cortical tubers and SENs can be detected *in utero* at mid to late gestation [14]. Moreover, immunohistochemical analyses have shown that cells within tubers and SENs express markers of multipotent radial glial cells (RGCs) at 20 weeks gestation and have activated mTOR signalling [15,16]. Thus, the origin of the neurological manifestations in TSC patients may coincide with the onset of neurogenesis in the developing foetal brain.

2.2 mTOR pathway activation in TSC

Through use of *Drosophila* models, a land mark discovery uncovered that the *dTSC1* and *dTSC2* genes regulate cell and whole organism size [17]. Genetic epistasis analysis positioned both *dTSC1* and *dTSC2* downstream of the insulin-phosphatide inositol-3-kinase (PI3K) pathway and upstream of ribosomal protein S6 kinase 1 (S6K1). TSC2 was subsequently shown to be a direct substrate of Akt (also known as PKB) within the PI3K/Akt signalling pathway (Fig. 1) [18]. Furthermore, TSC1/TSC2 was found to repress mTOR complex 1 (mTORC1) to signal to downstream substrates that include S6K1 and eukaryotic 4E-binding protein 1 (4E-BP1) [19]. Together, TSC1 and TSC2 were found to function as a GAP towards GTP-bound Rheb to convert it into an inactive GDP-bound form. Rheb-GTP potently activates mTORC1, but the mechanism by which Rheb activates mTORC1 is currently unclear. Given that Rheb-GTP is sufficient to activate mTORC1 when supplemented to *in vitro* mTORC1 kinase assays, mTORC1 could be considered a downstream effector of Rheb [20]. As well as being downstream of Akt within the PI3K signalling pathway, TSC1/2 was also found to be negatively regulated by the mitogen-activated protein kinase (MAPK) pathway (Fig. 1) [21]. TSC2 is inhibited through direct phosphorylation by ribosomal S6 kinase (RSK) within the MAPK signalling pathway [22]. This work collectively positions TSC1/2 at a converging point of two major signalling pathways, PI3K/Akt and MAPK/RSK, that can control cell growth control through mTORC1.

2.3 mTORC1

mTORC1 consists of three core components, the serine/threonine protein kinase mTOR, rapamycin-associated protein of TOR (Raptor) and Lst8 (also known as GbetaL) (Fig. 1) [23]. Raptor functions as a protein scaffold that interacts with downstream substrates that typically contain mTOR signalling (TOS) motifs that facilitates their phosphorylation by mTOR [20]. As well as substrate recognition, Raptor is also essential for recruitment of mTORC1 to the lysosome in a nutrient sensitive manner. In the presence of nutrients (especially leucine), Raptor binds to Rag small G-protein heterodimers and is then translocated to the Ragulator complex, which resides on lysosomal membranes [24]. Rheb is C-terminally prenylated, enabling tethering of Rheb to the lysosomal membrane, and this prenylation moiety is necessary for mTORC1 activation [25].

mTORC1's central role is to drive anabolic growth through the regulation of many cellular processes, including protein synthesis, lipogenesis, ribosomal biogenesis, and mitochondrial biogenesis (Fig. 1), for a recent review see Dodd and Dunlop [26]. mTORC1 is historically known as a master regulator of protein translation through direct phosphorylation of both S6K1 and 4E-BP1. After being activated by mTORC1, S6K1 associates with ribosomes and phosphorylates an array of translation initiation factors, which increases the efficiency of protein translation [27]. An S6K1 substrate with a known role in translation initiation is eIF4B, which associates with and enhances the RNA helicase activity of eIF4A to unwind the secondary structure in the 5'-untranslated region of mRNAs [27]. mRNA unwinding through eIF4A/4B allows for the rapid scanning of the ribosomes to the start codon and more efficient translation. 4E-BP1 binds to and represses eukaryotic initiation factor 4E (eIF4E) on the m⁷-cap moiety found on the 5'-end of mRNA, to negatively regulate protein translation. As well as directly regulating protein translation, mTORC1 indirectly enhances protein translation through increasing ribosomal biogenesis. mTORC1 does this through enhancing rRNA and ribosomal protein gene expression, which is dependent on S6K1 [28]. Through S6K1, mTORC1 also enhances *de novo* synthesis of pyrimidines, which are usually limiting for highly proliferative cells, by phosphorylation of CAD (carbamoyl-phosphate synthetase 2, aspartate transcarbamoylase, and dihydroorotase) [29]. The central role of mTORC1 signalling in so many cellular processes has stimulated neuroscientists to investigate the function of this pathway in neurogenesis.

2.4 mTORC2

mTOR also exists as a second complex, called mTOR complex 2 (mTORC2), which consists of rapamycin-insensitive companion of mTOR (Rictor), Lst8, and stress-activated protein kinase interacting protein 1 (SIN1). Through Rictor, mTORC2 has a different set of downstream substrates that control cell growth and proliferation. mTORC2 was discovered as the kinase responsible for phosphorylating and activating Akt/PKB on S473 [30]. Other major substrates of mTORC2 are serum glucocorticoid-induced protein kinase (SGK) [31] and protein kinase C isoforms [32]. mTORC2 is also necessary for controlling cytoskeletal dynamics, through regulation of actin polymerisation and has been shown to be necessary for long-term memory [4]. However, there is no evidence so far that mTORC2 signalling is involved in neurogenesis.

3 mTOR signalling and neurogenesis

3.1 Neurogenesis in the developing brain

Beginning around embryonic day (E) 8 in the mouse embryo neuroepithelial cells (NECs), derived from the neuroectoderm, rapidly proliferate through symmetrical cell division to dramatically increase the pool of neural progenitors. In neocortical development, these NECs then line the walls of the lateral ventricle as the neural tube closes around E9. The NECs, which up to this point have formed a pseudostratified neuroepithelium, then begin to transform into RGCs [33]. RGCs divide asymmetrically in the ventricular zone (VZ) to self-renew and generate intermediate progenitor cells (IPCs), or in around 10–20% of cases, postmitotic neurons (Fig. 2) [34]. IPC processes do not contact the ventricular or pial surfaces and over the course of development take up more basal positions to create the

subventricular zone (SVZ). IPCs divide symmetrically to either produce two post-mitotic neurons, or two additional IPCs before generating neurons [33]. IPCs are thus transit amplifying cells (TACs) that facilitate the huge increase in neuronal numbers that characterise cortical development. Finally, neuroblasts, which retain the potential to divide, migrate to their final position, before extending processes and terminally differentiating become to post-mitotic neurons (Fig. 2).

3.2 mTOR pathway activity in the central nervous system (CNS)

Expression analysis suggests that mTORC1 signalling is active in neurogenic niches in the developing mouse CNS. Phosphorylated forms of mTOR, S6K1, rpS6 and 4E-BP1 are strongly expressed at the ventricular surface at E13.5 [35]. Moreover, phospho-S6K1 is strongly expressed in RGCs at E13.5 and post-natally [35,36]. Direct evidence for the importance of mTORC1 signalling in brain development was first discovered with the isolation of the 'flat-top' mutant in a genetic screen for defects in mouse forebrain development [37]. Flat-top embryos completely fail to develop the telencephalon. The mutation responsible for the flat-top phenotype was originally mapped to FKBP-12-rapamycin associated protein (FRAP), which is now known as mTOR [38].

3.3 NSC proliferation and mTOR signalling

The requirement for mTOR in NSC proliferation *in vitro* has only been explored relatively recently. Primary neural stem/progenitor cells derived from E14.5 mouse embryos stimulated with EGF and FGF2, have reduced proliferation when treated with rapamycin [39]. Moreover, proliferation of neural progenitors in the ciliary marginal zone of the *Xenopus* retina is reduced by mTOR inhibition [40].

mTOR null mice die around E6 [41,42] and so cannot be used to dissect the role of this pathway in neural cell proliferation *in vivo*. However, a study using an *mTOR* conditional allele provided evidence of a role for mTOR in NSC proliferation. Ka et al. used a floxed *mTOR* allele crossed to a Nestin-cre expressing line to eliminate mTOR expression in NSCs [35]. These mice had microcephaly and a significant reduction in cortical thickness at E15.5, resulting from a reduction in both cell proliferation and cell size. Using a combination of BrdU labelling and staining for the proliferative marker Ki67 in the VZ, they found that loss of mTOR increased cell cycle length and decreased cell cycle re-entry at E15.5. They also found a 50% reduction in Tbr2 positive IPCs and a 67% decrease in Tbr2 and Ki67 double-positive proliferating IPCs in mTOR mutant brains. Loss of mTOR also resulted in a significant decrease in the number of post-mitotic cortical layer neurons.

Cloetta et al. used a floxed allele of *raptor* with Nestin-cre to specifically inhibit mTORC1 signalling in NSCs and also observed a microcephaly phenotype [43]. Similar to loss of mTOR, *raptor* mutant brains contained fewer cells with reduced cell size. Quantitation and positional analysis of BrdU labelled cells in the VZ showed aberrant localisation and reduced numbers of proliferating cells throughout the cortical column at E15.5, but not at E19.5. In contrast to the mTOR mutant mice, cell cycle length and re-entry were unaffected at E15.5 in the *raptor* mutant, but significantly reduced at E17.5 and E19.5, suggesting that glial progenitor cell proliferation, rather than neural progenitor proliferation was affected.

Moreover, the overall organisation of the *raptor* mutant cortex and distribution of cortical post-mitotic neurons appeared normal at E19.5, although the cortical layers were narrower than in controls. Thus, loss of *raptor* in NPCs has much weaker effects than loss of mTOR on neural progenitor proliferation, resulting in a less severe disruption of cortical development. This disparity may be due to additional inactivation of mTORC2 signalling in the *mTOR* mutant mice, or to differences in the strength of inhibition of signalling downstream of mTORC1.

To further elucidate the precise role of mTOR in NSCs Hartman et al. used an elegant *in vivo* electroporation approach enabling the tracking of neural lineages derived from single NSCs [36]. The mTOR activator Rheb, or the mTORC1 component Raptor, were knocked-down in NSCs by *in utero* electroporation of short hairpin RNAs into the lateral ventricle of postnatal day (P) 0 mice, together with a fluorescent reporter to track the electroporated cells. In keeping with the results of the *mTOR* conditional mutant, Rheb or Raptor knock-down caused a reduction in the number of labelled neurons in the olfactory bulb two weeks after electroporation and a reduction in NSC soma size. Interestingly though, three days after electroporation, although the number of Mash1 positive TACs and the number of Ki67 positive cells were both strongly reduced, there was no change in the number of TACs expressing proliferative markers. Gain of function studies, using a constitutively active allele of Rheb, showed that NSC proliferation was not increased three days after electroporation, but the number of TACs was double that of the control. These experiments are very informative as they suggest that the role of mTOR signalling in neurogenesis is to drive the differentiation of TACs from NSCs, rather than to regulate the cell cycle. Surprisingly, conditional knock-out of *Rheb1* using Nestin-Cre does not cause gross alteration of embryonic brain development or microcephaly, but brain weight and cortical thickness are reduced postnatally [44].

Activation of mTOR signalling, through conditional deletion of *Tsc1* or *Tsc2*, in neurons or neural progenitors, has consistently been shown to cause megalencephaly and increased cortical thickness [45]. Activation of mTOR signalling in RGCs, by conditional deletion of *Tsc2* using hGFAP-Cre (beginning at E13.5), did not alter BrdU incorporation in the SVZ [46]. However, there was a significant increase in the number of Tbr2 positive IPCs and a decrease in the number of post-mitotic neurons. Conditional deletion of *Tsc1* in neuroepithelial cells using Emx1-Cre (at E10.5) caused an increase in RGCs and IPCs in the SVZ and decreased post-mitotic neurons in several cortical layers at E16.5 [47]. Taken together these studies support the findings of Hartman et al. (2013), that activation of mTORC1 signalling in NPCs causes premature progenitor cell differentiation, rather than increased proliferation [36]. Thus, the megalencephaly and increased cortical thickness caused by activation of the mTOR pathway may result from aberrant differentiation, combined with increased soma volume, rather than an increase in the rate of proliferation of progenitor cells.

3.4 mTOR signalling and regulation of neuronal migration

The presence of giant cells that express doublecortin and LIS-1, markers of migrating neuroblasts, as well as white matter abnormalities adjacent to tubers, have suggested that

aberrant migration may be important in TSC neuropathology [48]. Correspondingly, ectopic neurons have been observed in the cortex and hippocampus in several *Tsc* gene conditional mutant mouse models, suggesting aberrant migration of neural progenitors [45]. Activation of mTORC1 signalling in NSCs in the postnatal SVZ by conditional removal of *Tsc1*, or expression of a constitutively active form of Rheb, causes failure of neuroblast migration to the olfactory bulb and the formation of heterotopias (clusters of misplaced cells) [49–51]. Strong defects in migration are also caused by knock-down of the mTOR pathway inhibitor RPT801/REDD1 in the embryonic rat VZ [52].

Migration and differentiation of neural progenitors are intimately linked and are difficult to extricate through analysis of fixed tissue. Importantly therefore, live imaging of slice cultures has shown that the migration speed of *Tsc1* mutant neuroblasts is significantly reduced [49]. Live imaging of neuroblasts with activated mTORC1 signalling in the postnatal rostral migratory stream, through loss of *Pten*, supports this finding, at least in part. Postnatal deletion of *Pten* resulted in a mixed population of neuroblasts that either had normal bipolar morphology and normal migration, or lost their polarity and were static [53]. mTORC1 pathway activation therefore acutely affects the migration of neural progenitors in the brain.

To investigate the effects of loss of mTOR in neuroblast migration, *in utero* electroporation of Dcx-cre-iGFP at E13.5 was used to express Cre in neural progenitors in floxed conditional mTOR mutant mice [35]. GFP labelled neurons analysed at P10 showed a normal pattern of migration into the cortical plate. Use of an independent Cre (Nex-cre) to delete mTOR in NPCs resulted in a normal pattern of localisation of Tbr1 and Cux1 positive neurons, implying that neuroblast migration was also unaffected in this paradigm. This study suggests that mTOR is not required for migration, at least of radially migrating neurons in the cortex. Thus, although activation of mTORC1 signalling is sufficient to perturb neuronal migration (Fig. 2), mTOR may not be required for migration.

3.5 Temporal regulation of neuronal differentiation by mTORC1

Histopathological analysis of giant cells and tubers in TSC patient tissue led to the hypothesis that aberrant neural cell differentiation contributes to TSC neuropathology [48,54–56]. The mTOR pathway was first discovered to regulate neuronal differentiation in the developing *Drosophila* eye [57–59]. Bateman and McNeill showed that activation of mTORC1 signalling in cells mutant for *Tsc1* or *Pten* causes precocious photoreceptor differentiation, whereas mTORC1 inhibition, in *insulin receptor* or *Rheb* mutant cells, causes a severe delay in photoreceptor differentiation [57]. Interestingly, mTOR signalling only regulates the differentiation of a subset of photoreceptor neurons, suggesting that differentiation is controlled by a specific developmental program, rather than as a general consequence of altered growth rate [59]. This suggestion was later shown to be true through the identification of novel factors that act downstream of mTORC1 which regulate neuronal differentiation (see Section 5) [60]. Loss of *Tsc1* in the developing mouse eye has recently also been shown to cause precocious differentiation and aberrant radial migration of retinal ganglion cells [61].

Experiments in vertebrate models have also provided strong evidence that mTORC1 signalling has a crucial role in regulating the timing of neuronal differentiation. The rate of neuronal differentiation from primary NPCs isolated from neonatal rat telencephalon tissue is significantly increased by stimulation with insulin and inhibited by rapamycin [62]. In chick, treatment of neural tube explant cultures with the PI3K inhibitor LY294002, or rapamycin, for 24 hours caused a reduction in the number of HuC/D expressing neurons [63]. Rapamycin soaked beads implanted into the developing neural tube in whole chick embryos also caused a local reduction in the number of neurons after 24 hours. This study demonstrates that active mTOR signalling is necessary for the early stages of neural progenitor differentiation in the developing neural tube.

Using a conditional *Tsc1* mutant mouse model with *Emx-cre*, Magri et al. isolated NSCs from E16.5 embryos and expanded these *in vitro* [47]. When induced to differentiate *in vitro* by growth factor withdrawal, *Tsc1* mutant cells gave rise to a much higher frequency of Tuj1 positive neurons. Conversely, treatment of wild-type NSCs with rapamycin completely prevented differentiation into neurons. *In vivo* studies using transgenic mice also strongly support a role for mTORC1 in neuronal differentiation (see Section 3.3). Therefore, a fundamental role of mTOR signalling in nervous system development is the temporal control of neuronal differentiation (Fig. 2).

4 mTOR signalling and neurogenesis in epilepsy

4.1 Aberrant neurogenesis in animal models of epilepsy

Epilepsy is a group of neurological disorders characterised by recurrent seizures [64]. Temporal lobe epilepsy (TLE) is the most common form of epilepsy in humans and is characterised by locally occurring seizures that may spread throughout unilateral or bilateral networks [64]. Studies of acute TLE animal models, using either electrical stimulation or administration of chemical convulsants, have shown dramatic alterations in neurogenesis in the SVZ and the subgranular zone of the hippocampus [65].

These studies are important, but do not address the key question of whether aberrant neurogenesis causes, or even contributes to seizures in these epilepsy models. A recent study by Cho et al. used an elegant genetic strategy to test the hypothesis that aberrant neurogenesis contributes to seizures and the cognitive deficits associated with TLE [66]. This study used mice that expressed a modified herpes simplex virus thymidine kinase (delta-HSV-TK) in NSCs using the nestin promoter. Subcutaneous administration of ganciclovir inducibly ablates proliferating NSCs in these mice, because ganciclovir is specifically phosphorylated by HSV-TK and acts as a toxic analogue of thymidine [67]. In a chemical convulsant (pilocarpine) mouse model of TLE, ablation of neurogenesis caused a 40% reduction in seizure frequency, but did not affect seizure duration or severity. Remarkably, the reduced seizure frequency was still apparent even after the mice were aged to around one year. Cho et al. also found that ablation of hippocampal neurogenesis rescued novel location recognition and novel object recognition hippocampus-dependent memory functions in TLE mice. Therefore, loss of hippocampal neurogenesis does not stop the occurrence of seizures, but does reduce their frequency, even over the long term, and also alleviates the associated impaired memory function. Neurogenesis thus contributes to

epileptogenesis in this model of TLE, but additional factors are involved in consolidating the seizure phenotype.

4.2 mTOR pathway activation in epilepsy models

The prevalence of seizures in TSC has stimulated investigation of the mTOR pathway in chemical convulsant animal models of epilepsy. In a kainate induced rat model of TLE mTOR activity, determined by phospho rpS6 expression, was observed to increase almost immediately in both the hippocampus and the neocortex [68]. Interestingly, there was also a second period of mTOR activation, starting at three days and peaking at five days after kainate injection, which returned to baseline after five weeks. This second peak was observed in the hippocampus, but not in the neocortex, suggesting that mTOR activation is associated with hippocampal epileptogenesis in this epilepsy model. Treatment with rapamycin also decreased the frequency of seizures seven weeks after kainate injection. Other studies have independently observed a similar pattern of mTOR pathway activation after chemical convulsant induced status epilepticus, or using a genetic model of absence epilepsy in rats [69–71]. Together these animal studies strongly support a link between mTOR activation and hippocampal epileptogenesis. Epileptogenesis can also occur through activation of mTORC1 signalling in the adult brain in the absence of altered neurogenesis, as acute biallelic *Tsc1* deletion in adult mice resulted in epilepsy within a few days [72].

4.3 mTOR pathway activation in human epilepsy syndromes

Rare conditions caused by mutations in mTOR pathway genes other than *TSC1* and *TSC2* are also associated with epilepsy. Polyhydramnios, megalencephaly, and symptomatic epilepsy (PMSE) syndrome is a rare neurodevelopmental disorder caused by a mutation in the *STRADA* gene [73]. STRADA binds to and activates the kinase LKB1, which phosphorylates TSC2 and so PMSE patients have increased mTOR pathway activity [74].

Mutations in the GATOR1 (GAP activity towards rags subcomplex 1) component DEPDC5 (disheveled, Egl-10 and pleckstrin domain containing protein 5) were identified in patients with a rare inherited form of focal epilepsy. Subsequent genetic screening showed that 12–37% of patients with common nonlesional focal epilepsy also have mutations in *DEPDC5* [75,76]. However, the prevalence of *DEPDC5* mutations in one study of TLE was much lower (2.2%) [77]. Thus, mTOR pathway activation due to mutations DEPDC5 may be a common cause at least of idiopathic focal epilepsy.

Focal cortical dysplasia (FCD) and hemimegalencephaly (HME) are two common types of malformations of cortical development. Using next generation sequencing methods to compare mutations present in blood versus brain tissue, Lim et al. recently identified somatic mutations in *mTOR* from brain tissue in 15.6% of FCD type II patients [78]. When expressed in cells, these mutations activated mTOR, as assessed by phospho-S6K1 expression. Moreover, using *in utero* electroporation, one of the recurrent mutations (L2427P) was shown to inhibit migration of neuroblasts into the cortical plate. Mice electroporated with the L2427P mTOR mutant also exhibited spontaneous seizures postnatally. Other recent studies have confirmed that somatic activating mutations in mTOR account for a minor proportion of FCD type II [79,80]. An inherited mutation in *DEPDC5*

has also been identified in two brothers with FCD type II [81]. These studies demonstrate that the cortical lesions in TSC and FCD share a common molecular basis, at least in a subset of FCD type II patients.

Similar efforts using exome sequencing from blood and resected brain tissue enabled Lee et al. to identify *de novo* somatic mutations in *PIK3CA*, *AKT3* and *mTOR* genes in the brains of HME patients [82]. Cytomegalic neurons in the brains of these patients expressed high levels of phospho-S6K1, suggesting that the identified mutations activate the mTORC1 pathway. A recent study also found germline or somatic mutations in *PIK3CA*, *mTOR* and also in *TSC2* and *DEPDC5* in HME patients [83]. Interestingly, exome sequencing also identified a *de novo* mutation in *mTOR* in a patient with the epileptic encephalopathy Lennox–Gastaut syndrome [84]. This patient had no detectable structural brain malformations, suggesting that the mTOR pathway can be associated with types of epilepsy that do not cause large scale alterations in cortical development.

Taken together, these patient studies demonstrate that mTOR activation contributes to a small but significant proportion of epilepsy syndromes. Thus, the aberrant neurogenesis associated with activation of mTOR signalling may contribute to these diseases.

5 Novel mTOR pathway factors regulating neurogenesis

Evidence from a variety of model systems has shown a key role for mTOR signalling in neural development, specifically in neuronal differentiation and migration (see Section 3). A fundamentally important aim is therefore to identify the mechanism by which mTORC1 signalling regulates neurogenic processes. An *in vivo* RNAi screen in the developing *Drosophila* eye recently identified the first factors acting downstream of mTORC1 that regulate neuronal differentiation. The proteins Unkempt (Unk) and Headcase (Hdc) form a complex and through this screen were identified as negative regulators of photoreceptor differentiation acting downstream of mTORC1 [60]. The Unk/Hdc complex uncouples the role of mTORC1 signalling in neuronal differentiation and growth control (Fig. 3). Moreover, knock-down of Unk in the mouse embryonic SVZ causes defects in neuroblast migration and morphology [85]. Thus, Unk has a conserved role in nervous system development. Investigation of novel mTOR pathway components regulating neurogenesis, such as Unkempt, will provide insight into the mechanism by which mTOR regulates neural development. This understanding could also lead to the development of more specific targeted therapies for the neurological manifestations of diseases like TSC and FCD.

6 Conclusions and future directions

Research during the last ten years has revealed fundamentally important roles for mTOR signalling in nervous system development and led to new insight into the neurological manifestations of TSC. Although the details are not yet fully understood, it is clear that the mTOR pathway is a key player in neuronal differentiation and migration. Future studies will build on this knowledge to characterise the factors that act downstream of mTOR and how this pathway integrates with the multitude of other influences in the neurogenic niche.

Relatively little is known about the role of mTOR signalling in gliogenesis. Available evidence from *Drosophila* and mouse models suggests the *TSC* genes and mTORC1 signalling regulate the proliferation and morphogenesis of glia [86–88]. Future studies of the role of mTORC1 signalling in neurogenesis and gliogenesis will benefit not only our understanding of TSC, but also of an emerging cadre of other neurological diseases caused by mTOR pathway activation.

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Abbreviations

CNS	central nervous system
FCD	focal cortical dysplasia
HME	hemimegalencephaly
IPC	intermediate progenitor cell
mTORC	mechanistic target of rapamycin complex
NSC	neural stem cell
RGC	radial glial cell
SEGA	subependymal giant cell astrocytoma
SEN	subependymal nodule
SVZ	subventricular zone
TAC	transit amplifying cell
TLE	temporal lobe epilepsy
TSC	tuberous sclerosis complex
VZ	ventricular zone

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Fig. 1.

An overview of mTORC1 signalling. See Sections 2.2 and 2.3 for details.

Fig. 2. Neurogenic processes regulated by mTORC1 signalling. Processes regulated by mTORC1 signalling are indicated by red arrows. See Section 3 for details.

Fig. 3.

A model for the regulation of neuronal differentiation by the Unkempt/Headcase complex acting downstream of mTORC1. See Section 5 for details.