Caveat mTOR: aberrant signaling disrupts corticogenesis

Lucy R. Osborne

Commentary

The mammalian target of rapamycin (mTOR) signaling pathway is activated in several disorders associated with benign tumors and malformations of the cerebral cortex. In this issue of the *JCI*, Orlova et al. have now definitively added another disorder to this group by demonstrating that activation of mTOR signaling is associated with polyhydramnios, megalencephaly, and symptomatic epilepsy syndrome (PMSE), which is characterized by severe intractable epilepsy and megalencephaly. PMSE is caused by lack of the pseudokinase STE20-related kinase adaptor α (STRADα), and Orlova et al. show that reduction of STRADα levels during corticogenesis in the mouse results in a cellular phenotype and neuronal migration defects similar to those observed in patients with PMSE, clearly demonstrating a pivotal role for STRADα in cell polarity and growth. This study helps pave the way for possible therapeutic intervention with rapamycin to control the epilepsy and learning disabilities associated with this disorder.

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Caveat mTOR: aberrant signaling disrupts corticogenesis

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The mammalian target of rapamycin (mTOR) signaling pathway is activated in several disorders associated with benign tumors and malformations of the cerebral cortex. In this issue of the *JCI*, Orlova et al. have now definitively added another disorder to this group by demonstrating that activation of mTOR signaling is associated with polyhydramnios, megalencephaly, and symptomatic epilepsy syndrome (PMSE), which is characterized by severe intractable epilepsy and megalencephaly. PMSE is caused by lack of the pseudokinase STE20-related kinase adaptor α (STRADα), and Orlova et al. show that reduction of STRADα levels during corticogenesis in the mouse results in a cellular phenotype and neuronal migration defects similar to those observed in patients with PMSE, clearly demonstrating a pivotal role for STRADα in cell polarity and growth. This study helps pave the way for possible therapeutic intervention with rapamycin to control the epilepsy and learning disabilities associated with this disorder.

Development of the human cortex is a complex and finely orchestrated process. The layers of the developing neocortex are generated through neuronal migration, whereby neurons travel from their place of origin in the proliferating ventricular zone to their final position in the brain. This cortical lamination is generated from the inside out, with the neurons that contribute to each layer traveling past the existing layers; perturbation of this carefully controlled neuronal migration can result in heterotopia (neurons in the wrong place) and dysplasia (disorganization of the normal structure of the cortex) that disrupt the normal cortical circuitry. Imbalance between excitatory and inhibitory systems in the cortex can lead to spontaneous electrical discharge with catastrophic consequences, often in the form of intractable or medication-resistant epilepsy and severe intellectual disability (1).

Polyhydramnios, megalencephaly, and symptomatic epilepsy syndrome (PMSE), recently described in an Old Order Mennonite population, is associated with craniofacial dysmorphism (large forehead, widely spaced eyes, and large mouth), an abnormally large brain, and severe, early-onset intractable epilepsy (2). PMSE in this kindred is caused by homozygous deletion of a portion of the STE20-related kinase adaptor α gene (*STRADA*; encoding STRADα) on human chromosome 17 (2). STRADα normally binds and exports the protein kinase serine/threonine kinase 11 (STK11; also known as LKB1) out of the nucleus, where they bind to MO25 to form a trimeric complex that has an inhibitory effect on mammalian target of rapamycin (mTOR) signaling through the sequential phosphorylation of AMPK and the tuberous sclerosis complex 1/ tuberous sclerosis complex 2 (TSC1/TSC2) complex (Figure 1 and ref. 3). Aberrant activation of the mTOR pathway was identified in the brain of an individual with PMSE by the presence of high levels of phosphorylated ribosomal S6 protein (2), a downstream target of mTOR. This finding is suggestive of a link between the cellular mechanism underlying this disorder and that previously shown to be responsible for a group of syndromes characterized by hamartomas, benign tumors composed of differ-
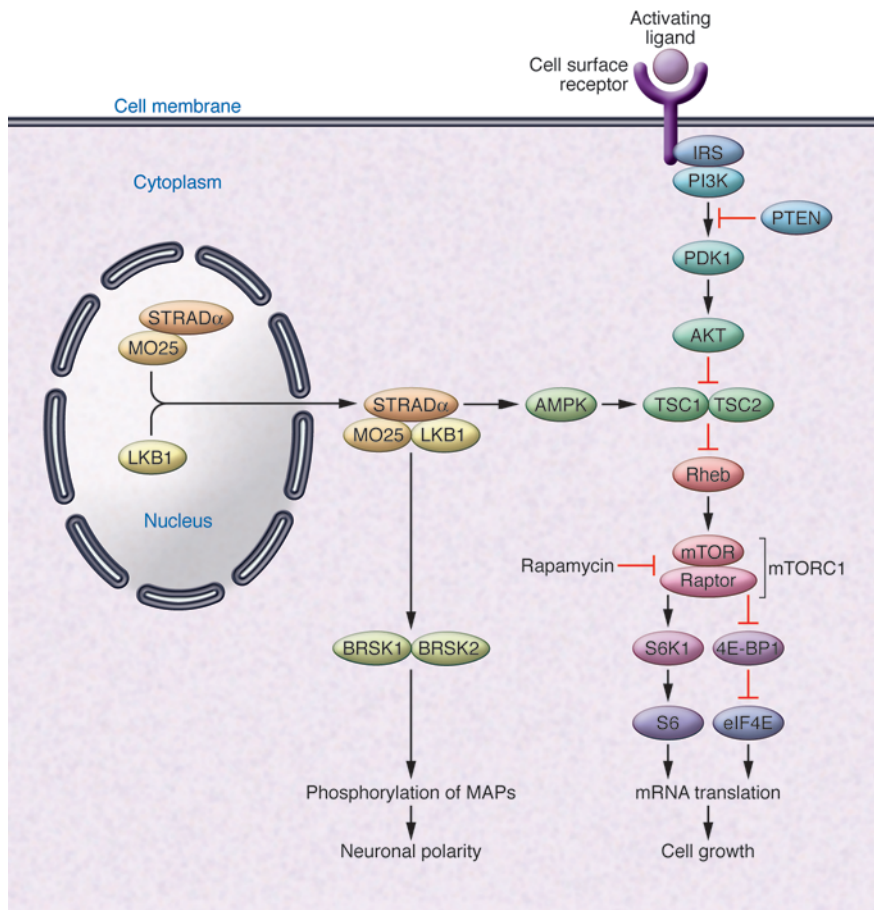
Figure 1
mTOR signaling pathway is involved in genetic syndromes. Shown is the relationship among different proteins encoded by genes known to be responsible for genetic syndromes associated with activation of mTORC1. Activation of mTORC1 ultimately leads to increased protein synthesis, and thereby increased cell growth. Germline mutations that result in mTORC1 hyperactivation are therefore associated with syndromes characterized by cellular overgrowth, usually in the form of hamartomas. LKB1 also activates an mTORC1-independent pathway that controls neuronal polarity specification; it is therefore possible that the neuronal migration defects seen in individuals with PMSE may be due to effects on this pathway, and not to hyperactivation of mTOR signaling. This would explain the lack of neuronal migration defects in disorders associated with germline mutations that do not affect LKB1 localization or function. 4E-BP1, eIF4E-binding protein 1; eIF4E, eukaryotic translation initiation factor 4E; MAP, microtubule-associate protein; PDK1, phosphoinositide-dependent protein kinase–1; Rheb, Ras homolog enriched in brain; S6K1, S6 kinase, 70 kDa, polypeptide 1.

entiated but disorganized cells that form in the tissue of their origin. In this issue of the *JCI*, Orlova et al. have now definitively linked PMSE with the mTOR signaling pathway and elucidated the cellular mechanism by which constitutive activation of the pathway occurs (4). Specifically, they found that loss of STRADα leads to activation of signaling by the mTOR complex 1 (mTORC1), a multiprotein complex that contains mTOR and regulatory associated protein of mTOR (raptor), because the trimeric STRADα/LKB1/MO25 complex no longer exerts its inhibitory effects on this pathway (Figure 1).

PMSE: a nonclassic hamartoma syndrome

Regulation of mTOR signaling is essential for controlled cellular growth and proliferation, since mTOR directly phosphorylates and thereby activates important translation initiation factors, acting as a master controller of protein synthesis (5). Numerous cancers exhibit aberrant mTOR signaling, but germline mutations of the genes encoding key upstream mTOR regulators

are associated with syndromes characterized by cellular overgrowth, usually in the form of hamartomas (6). Proteins that regulate the mTOR pathway therefore act as tumor suppressors, and germline mutations predispose to loss of heterozygosity and hamartoma formation (Table 1).

Because the genes that are mutated in hamartoma syndromes are functioning, at least in part, as classic tumor suppressors, we can presume that the pattern of benign growths in rapidly dividing epithelial tissues in which replication errors are most likely to occur reflects the loss of the remaining allele. The finding of Orlova et al. (4) that PMSE is also caused by dysregulation of mTOR signaling was, therefore, somewhat unexpected, since hamartomas are not a clinical feature of this syndrome. One explanation that Orlova et al. propose for the lack of tumors in patients with PMSE is that STRADβ, a pseudokinase closely related to STRADα, may also export LKB1 from the nucleus, albeit to a much lesser extent than STRADα. STRADβ is expressed throughout the gastrointestinal tract, but not in the ventricular zone of the developing

cortex, which suggests that it may be able to compensate for complete loss of STRADα in certain tissues, but not in others (7, 8). This could explain why in PMSE, aberrant cell growth is only seen in the brain (in the form of megalencephaly) and in certain craniofacial regions, such as the mandible and forehead (where it manifests as craniofacial dysmorphisms). Conversely, as Orlova et al. point out (4), neurological symptoms are not seen in individuals with the hamartoma syndrome Peutz-Jeghers syndrome (PJS), which is caused by germline mutations in *LKB1*. In PJS, however, 1 functional copy of *LKB1* remains, suggesting that a minimum threshold of LKB1 needs to be reached before clinical symptoms present. This idea is supported by the observation that heterozygous carriers of the PMSE-causing *STRADA* mutation show no apparent signs of the disease.

Cellular effects of mTOR dysregulation

Although the clinical presentation of PMSE differs from that of hamartoma syndromes, the characteristic cellular effects of mTOR

Table 1
Genetic disorders involving activation of the mTOR pathway

Disease	Gene	Clinical symptoms	OMIM no.
TSC	*TSC1*	Hamartomas in brain, kidney, skin, retina	191100
TSC	*TSC2*	Seizures, intellectual disability	613254
Cowden disease (including Lhermitte-Duclos syndrome)	*PTEN*	Hamartomas in skin, mucous membranes, breast, thyroid, and rarely brain; macrocephaly, vascular anomalies	158350
Bannayan-Riley-Ruvalcaba syndrome	*PTEN*	Hamartomas in gastrointestine, lipomas; macrocephaly, vacular anomalies	153480
Macrocephaly/autism syndrome	*PTEN*	Macrocephaly, autism, developmental delay, obesity	605309
PJS	*LKB1*	Hamartomas in gastrointestine; hyperpigmented macules on lips, oral mucosa, and hands	175200
PMSE	*STRADA*	Macrocephaly, severe epilepsy, developmental delay, hypotonia, craniofacial dysmorphism	611087

signaling are still present. Orlova et al. demonstrated cytomegaly, hyperphosphorylation of ribosomal protein S6 (S6), and increased expression of c-MYC, another downstream target of the mTOR signaling cascade, in postmortem brain tissue from an individual with PMSE (4). LKB1 nuclear localization was unique to PMSE, as might be expected, and was not seen in the hamartoma syndrome tuberous sclerosis complex (TSC) or in other cortical dysplasias. These cellular effects were seen not only in the brain of the individual with PMSE, but also in cultured mouse neural progenitor cells after knockdown of *Strada* for only a few days, indicating that the cells are particularly sensitive to changes in mTOR signaling.

Neurological symptoms in disorders associated with activation of the mTOR pathway are common, with the exception of PJS, and are thought to be caused by malformations of the cerebral cortex. These cortical malformations manifest in the form of cortical tubers, subependymal nodules, and giant cell astrocytomas in TSC and rare brain hamartomas in Cowden disease, but macrocephaly and cytomegaly are also common (6). In addition to cytomegaly, Orlova et al. found evidence of neuronal heterotopia in PMSE by studying postmortem brain tissue from an infant with the disorder and performing MRI on other affected individuals; their findings suggest that STRADα deficiency caused a defect in neuronal migration (4). The authors tested this hypothesis by knocking down *Strada* in the developing mouse cortex (E14) and found an observable defect in cortical lamination both 3 and 5 days later: cells expressing the *Strada*-specific shRNA knockdown construct failed to migrate properly within the cortex and became clustered in the ventricular zone.

A similar experiment using *Lkb1* RNAi knockdown had previously produced an equivalent arrest in neuronal migration, which was shown to be caused by loss of cell polarity in the transfected neurons (9). The majority of transfected neurons failed to exhibit axon specification, but a small subset that managed to migrate through the cortical plate showed inverted polarity. Conditional deletion of *Lkb1* in pyramidal neurons, the primary excitatory cells of the cortex, also results in cell polarity defects because of the inability of LKB1 to phosphorylate, and therefore activate, BR serine/threonine kinase 1 (BRSK1) and BRSK2, which are essential for neuronal polarity specification (8). Orlova et al. demonstrated that in cells lacking STRADα, nuclear export of LKB1 was dramatically impaired, likely seriously affecting its catalytic ability (4). This was in contrast to TSC and other cortical dysplasia syndromes, in which LKB1 localization was not altered (4). The authors therefore hypothesize that STRADα may have an LKB1-independent function that is responsible for cortical malformations in PMSE; intriguingly, recent data from *C. elegans* suggests that in this organism, the equivalent proteins to STRADα and LKB1 (STRD-1 and PAR-4, respectively) can regulate neuronal polarity through different effectors (although unlike vertebrates, *C. elegans* appears to have only a single STRAD isoform; ref. 10). However, the experimental data generated from the mouse, combined with the lack of documented neuronal migration defects in the hamartoma syndromes, perhaps indicate that the neuronal migration defects seen in PMSE may actually be due to effects on the distinct cell polarity pathway downstream of LKB1, not to activation of mTOR signaling. This would explain the lack of neuronal migration defects in disorders associated with proteins that do not affect LKB1 localization or function.

Potential efficacy of rapamycin in PMSE
The downstream effects of mTOR hyperactivation can be drastically altered with rapamycin, and many clinical trials in various forms of cancer associated with aberrant mTOR activation are currently ongoing. The potential for rapamycin therapy in disorders with germline mutations activating mTOR signaling is still unknown, although in TSC, it has been shown to induce regression of astrocytomas and is currently in clinical trial as a topical treatment for cutaneous lesions (11, 12). Successful treatment of the neurological effects in PMSE and other mTOR signaling disorders will be far more challenging, since they occur during the development and maturation of the cortex. Studies in mouse models have shown some promise, with decreased phosphorylation of the mTOR downstream target S6 and some reduction in cell hypertrophy and seizure activity in a mouse model of cortical dysplasia with phosphatase and tensin homolog (*Pten*) deletion in a subset of glutamatergic cortical neurons (13). In a second model of TSC with *Tsc1* deletion in mature astrocytes, rapamycin also improved seizure activity (14), and *Tsc2*$^{+/-}$ mice showed improvement in learning deficits when treated (15). Here, Orlova et al. showed that the cytomegaly associated with knockdown of *Strada* in mouse neuronal progenitors was completely alleviated with concurrent rapamycin treatment (4). It is therefore possible that rapamycin may counteract some of the ongoing cytomegaly and changes in neuronal morphology caused by activation of the mTOR pathway (16), although whether it will be

effective against the severe epilepsy seen in individuals with PMSE and TSC remains to be seen. In both disorders, the brain insult occurs early and may not be reversible, and in the case of PMSE, the severe seizures may be caused by a combination of neuronal migration defects and mTOR activation. In rat models of epilepsy, only continuous treatment with rapamycin could counteract seizure-induced formation of new projections from axon bundles in the hippocampal region of the brain, and existing axonal reorganization could not be reversed (17, 18).

Future directions

Although the present study by Orlova et al. (4) definitively adds PMSE to the growing list of disorders with aberrant activation of mTOR signaling, there are questions about the pathogenesis of PMSE that remain unanswered. The relative contributions of mTOR activation and impaired LKB1 signaling to cortical dysplasia will be key in determining potential routes for therapeutic intervention. Rapamycin treatment is one approach, but partial restoration of LKB1 function may also prove helpful. A *Strada* knockout mouse will be extremely helpful in further determining the pre- and postnatal role of STRADα in brain development and function, and will also provide a valuable tool for the testing of therapeutic agents in PMSE.

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