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Review

A circuitry and biochemical basis for tuberous sclerosis symptoms: from epilepsy to neurocognitive deficits

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ABSTRACT

Tuberous sclerosis complex (TSC) is an autosomal dominant monogenetic disorder that is characterized by the formation of benign tumors in several organs as well as brain malformations and neuronal defects. TSC is caused by inactivating mutations in one of two genes, *TSC1* and *TSC2*, resulting in increased activity of the mammalian Target of Rapamycin (mTOR). Here, we explore the cytoarchitectural and functional CNS aberrations that may account for the neurological presentations of TSC, notably seizures, hydrocephalus, and cognitive and psychological impairments. In particular, recent mouse models of brain lesions are presented with an emphasis on using electroporation to allow the generation of discrete lesions resulting from loss of heterozygosity during perinatal development. Cortical lesions are thought to contribute to epileptogenesis and worsening of cognitive defects. However, it has recently been suggested that being born with a mutant allele without loss of heterozygosity and associated cortical lesions is sufficient to generate cognitive and neuropsychiatric problems. We will thus discuss the function of mTOR hyperactivity on neuronal circuit formation and the potential consequences of being born heterozygous on neuronal function and the biochemistry of synaptic plasticity, the cellular substrate of learning and memory. Ultimately, a major goal of TSC research is to identify the cellular and molecular mechanisms downstream of mTOR underlying the neurological manifestations observed in TSC patients and identify novel therapeutic targets to prevent the formation of brain lesions and restore neuronal function.

Contents

Abbreviations: CNS, central nervous system; CreERT2, inducible Cre; CSF, cerebral spinal fluid; EEG, electroencephalography; 4E-BP1, eIF4E-binding protein 1; E, embryonic day; fl, floxed; FCDs, focal cortical dysplasias; *FMR1*, FMRP gene; FMRP, fragile X mental retardation protein; FXS, fragile X syndrome; GFAP, glial fibrillary acidic protein; GAP, GTPase activating protein; hgfap, human gfap; IUE, *in utero* electroporation; LV, lateral ventricle; LOH, loss of heterozygosity; LTD, long-term depression; LTP, long-term potentiation; MRI, magnetic resonance imaging; mTOR, mammalian Target of Rapamycin; mgfap, mouse gfap; mGluR-LTD, metabotropic glutamate receptor class I long term depression; mTORC1 or mTORC2, mTOR complex 1 or 2; P, postnatal day; PP2A, protein phosphatase 2A; RGCs, retinal ganglion cells; Rheb, Ras homolog enriched in brain; S6K1, p70 S6 Kinase 1; SEGA, subependymal giant cell astrocytoma; SEN, subependymal nodules; SEZ, subependymal zone; SynI-Cre, *Synapsin I* promoter-driven Cre; $Tsc1^{fl/fl}$, floxed *Tsc1* alleles (transgenic mice); $Tsc1^{fl/mut}$, floxed and mutant *Tsc1* alleles; $Tsc1^{wt/mut}$, wildtype and mutant *Tsc1* alleles; TSC, tuberous sclerosis complex; TSC1 or TSC2, TSC gene 1 or gene 2.

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1. Introduction

Tuberous sclerosis complex (TSC) is an inherited developmental disease characterized by discrete lesions in diverse tissues, including the skin, heart, kidney, lung, and brain (Crino et al., 2006). The incidence of TSC is estimated to be between 1:6000 and 1:10,000 individuals (O'Callaghan et al., 1998). TSC is caused by inactivating mutations in one of two genes, *TSC1* and *TSC2*, which encode for the proteins hamartin and tuberin, respectively (European Chromosome 16 Tuberous Sclerosis Consortium, 1993; van Slegtenhorst et al., 1997). Inactivating mutations in *TSC1* or *TSC2* subsequently lead to hyperactivity of the mTOR pathway (Kwiatkowski, 2003a,b). Most patients are born with at least one detectable mutation and are thus heterozygous for either *TSC1* or *TSC2*. Very often, there are subsequent inactivating mutations of the other functional allele (Green et al., 1994; Sepp et al., 1996). This process, known as loss of heterozygosity (LOH), occurs somatically in a subset of cells, and is often detectable within peripheral and brain lesions (Kwiatkowski and Manning, 2005; Tsai and Crino, 2012), but another mechanism leading to *TSC1* or *TSC2* haploinsuffiency or alteration in another component of the TSC pathway, such as inflammation or epigenetic alterations, may also occur, but these mechanisms need further investigations (Crino, 2013).

Although TSC affects many organ systems, the neurological symptoms (*i.e.*, seizures, mental retardation, autism, and hydrocephalus) account for the most significant mortality and morbidity (de Vries, 2010; Orlova and Crino, 2010). Seizures are observed in the vast majority of patients. They often begin during the first year of life as infantile spasms and are often unresponsive to conventional pharmacological interventions (Curatolo et al., 2012; Curatolo and Moavero, 2010). In addition, more than 50% of affected children exhibit mental retardation and cognitive delay, with many (~40%) exhibiting autistic traits (Curatolo et al., 2010; Greenstein and Cassidy, 1986; Weber et al., 2000). Presently, there are no known cures for TSC. However, thanks to an increasing understanding of the disease etiology, treatments are now on the horizon (Khwaja and Sahin, 2011).

This review explores the cytoarchitectural and functional CNS aberrations that may account for the neurological presentations of TSC, notably seizures, hydrocephalus, and cognitive and psychological impairments. In addition, this review expounds upon alterations that may be independent of gross anatomical disturbances, including changes in neuronal connectivity and plasticity that may account for cognitive and psychiatric impairments in TSC.

Following background on mTOR signaling and the genetics of the disease, approaches recapitulating the LOH-associated brain lesions will be presented. In particular, a combination of technical approaches is being used to recapitulate cortical and subcortical lesions. Most notably, *in utero* and neonatal electroporation are discussed in relation to identifying defects in neuronal positioning, morphogenesis, and functional connectivity. The next sections deal with the identification of cellular and molecular correlates of cognitive and psychiatric deficits that may arise independent of neurological lesions. In particular, the effect of TSC1/2 dysfunction on integration, connectivity, and plasticity, and the interaction of the TSC-mTOR signaling cascade with another key autism-related pathway may provide mechanistic insights into novel therapies.

2. TSC-mTOR signaling

TSC1, TSC2, and TBC1D7 form a heteromeric complex that can bind to and stimulate the GTPase Ras homolog enriched in brain Rheb (Dibble et al., 2012; Inoki et al., 2003; Nakashima et al., 2007; Zhang et al., 2003; for reviews see Kwiatkowski and Manning, 2005; Tee et al., 2002) (Fig. 1). The heteromeric complex functions as a GTPase Activating Protein (GAP), which drives Rheb from an active GTP-bound state to an inactive GDP-bound state. Active Rheb directly activates the mTOR kinase by altering substrate affinities (Sato et al., 2009). Thus, the GAP complex acts as a negative regulator of Rheb and thus mTOR. mTOR is a shared component of two complexes, mTORC1 and mTORC2 (Laplante and Sabatini, 2012a). These two biochemically distinct complexes vary in their downstream substrates; however, the actions of the TSC GAP are predominantly linked to mTORC1 signaling (Laplante and Sabatini, 2012b). mTORC1 is thought to regulate protein translation through the direct phosphorylation of eIF4E-binding protein 1 (4E-BP1) and p70 S6 Kinase 1 (S6K1), which phosphorylates the ribosomal protein S6. Activation of both 4E-BP and S6K1 are required for appropriate growth factor-dependent translation of mRNA transcripts and cell growth (Hentges et al., 2001).

In TSC, canonical inactivating mutations in *TSC1* or *TSC2* result in hyperactivation of mTORC1, constitutive phosphorylation of 4E-BP1, and activation of ribosomal protein S6 through S6K1 phosphorylation. The result is sustained translation of growth-promoting transcripts. Despite the fact that several non-canonical pathways are activated as well, inhibition of mTORC1 through rapamycin, as detailed below, is sufficient to reverse nearly all phenotypes in animal models. As a result, the contribution of mTORC2 has not been extensively explored in regards to TSC. However, like any drug, rapamycin is imperfect and could potentially, depending on dose and length of treatment, result in mTORC2 inhibition. Regardless, mTORC1 would appear to be the primary target of the TSC GAP. Finally, it remains unaddressed whether mTORC2 inhibition may also reverse cellular phenotypes seen in TSC models and which mTORC1 (noted mTOR throughout the review) substrates are required for each respective cellular process.