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Metabotropic glutamate receptor-dependent long-term depression is impaired due to elevated ERK signaling in the ΔRG mouse model of tuberous sclerosis complex

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ABSTRACT

Tuberous sclerosis complex (TSC) and fragile X syndrome (FXS) are caused by mutations in negative regulators of translation. FXS model mice exhibit enhanced metabotropic glutamate receptor-dependent long-term depression (mGluR-LTD). Therefore, we hypothesized that a mouse model of TSC, ΔRG transgenic mice, also would exhibit enhanced mGluR-LTD. We measured the impact of TSC2-GAP mutations on the mTORC1 and ERK signaling pathways and protein synthesis-dependent hippocampal synaptic plasticity in ΔRG transgenic mice. These mice express a dominant/negative TSC2 that binds to TSC1, but has a deletion and substitution mutation in its GAP-domain, resulting in inactivation of the complex. Consistent with previous studies of several other lines of TSC model mice, we observed elevated S6 phosphorylation in the brains of ΔRG mice, suggesting upregulated translation. Surprisingly, mGluR-LTD was not enhanced, but rather was impaired in the ΔRG transgenic mice, indicating that TSC and FXS have divergent synaptic plasticity phenotypes. Similar to patients with TSC, the ΔRG transgenic mice exhibit elevated ERK signaling. Moreover, the mGluR-LTD impairment displayed by the ΔRG transgenic mice was rescued with the MEK–ERK inhibitor U0126. Our results suggest that the mGluR-LTD impairment observed in ΔRG mice involves aberrant TSC1/2-ERK signaling.

Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder that can cause hamartomas, benign and malignant neoplasms, seizures, mental impairment and autism (DiMario, 2004). At the molecular level, TSC is caused by either the loss or malfunction of either hamartin (TSC1) or tuberin (TSC2), which interact in a heterodimer known as the TSC1/TSC2 complex, to negatively regulate mammalian target of rapamycin complex 1 (mTORC1) (Cheadle et al., 2000). mTORC1 functions as a molecular gatekeeper for cap-dependent translation initiation in neurons. Activation of the phosphoinositide 3-kinase (PI3K)/Akt and extracellular signal-regulated kinase (ERK) signaling pathways results in the phosphorylation of TSC2 and inhibition of the GTPase-activating protein (GAP) activity of TSC2, which leads to increased levels of Rheb-GTP. This type of signaling activates the mTOR complex 1 (mTORC1) and subsequent phosphorylation ribosomal S6 kinase 1 (S6K1) and eukaryote initiation factor 4E-binding protein (4E-BP), key translation initiation regulators (Cai et al., 2006; Jozwiak, 2006; Jozwiak et al., 2005; Orlova and Crino, 2010; Yang et al., 2006).

It has been estimated that sporadic cases of TSC range from 60 to 70% of the cases reported, and that *TSC1* mutations are significantly underrepresented compared to *TSC2* (Jones et al., 1997). *TSC2* gene mutations are more frequent and result in a more severe phenotype in TSC patients (i.e. seizures and learning disability), with the exception of reported cases of patients with no mutation identified, as well as one *TSC2* mutation that causes a more mild phenotype (Camposano et al., 2009; Dabora et al., 2001; Jansen et al., 2006; Kwiatkowski, 2003). In addition, the *TSC2* gene is more prone than the *TSC1* gene to large deletions, rearrangements, and missense mutations. Of particular interest is the finding that missense mutations are clustered within *TSC2* exons 34–38, which encode for either Rap1GAP or GAP3 (Maheshwar et al., 1997). The TSC2-GAP domain is an essential structural domain for the hydrolysis of GTP-bound Rheb to its inactive GDP-bound form (Tee et al., 2003).

Studies have shown that either loss or malfunction of TSC1 and TSC2 usually results in activation of S6K1 and enhanced ribosomal protein S6 phosphorylation, resulting in defective regulation of cell size and proliferation (Krymskaya, 2003; Uhlmann et al., 2004).

Abbreviations: 4E-BP, eIF4E binding protein; ACSF, artificial cerebrospinal fluid; CMV, cytomegalovirus; FXS, fragile X syndrome; GAP, GTPase-activating protein; GDP, guanosine diphosphate; GTP, guanosine triphosphate; mTORC1, mammalian target of rapamycin complex 1; Rheb, Ras homolog-enriched in brain; STEP, striatal-enriched tyrosine phosphatase; TSC, tuberous sclerosis complex.

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Moreover, studies in hippocampal pyramidal neurons have shown that the TSC pathway regulates soma size, the density and size of dendritic spines, and the properties of excitatory synapses, particularly AMPA receptor-mediated currents (Tavazoie et al., 2005). Additional studies have shown that loss of TSC1 function in the brain leads to neocortical hyperexcitability associated with increased glutamate-mediated excitation in both human tissue and mouse brain (Wang et al., 2007). Finally, TSC2 heterozygous knockout mice were shown to exhibit elevated hippocampal mTORC1 signaling, which led to abnormal long-term potentiation (LTP) and deficits in hippocampus-dependent memory (Ehninger et al., 2008).

The ΔRG transgenic mouse has been developed, carrying a deletion in TSC2 of amino acid residues 1617–1655 and a substitution of amino acid residues 1679–1742, which interferes with both the GAP domain and rabaptin-5 binding motif of TSC2, respectively (Govindarajan et al., 2005; Pasumarthi et al., 2000). As a result, this dominant/negative TSC2 protein is not able to hydrolyze GTP-bound small G-proteins, such as Rap1 and Rheb (Govindarajan et al., 2005; Pasumarthi et al., 2000; Zhang et al., 2003). Previous studies have shown that ΔRG transgenic mice have increased expression of the dominant/negative TSC2 driven by the cytomegalovirus (CMV) promoter and develop skin and brain abnormalities consistent with those observed in TSC patients (Bhatia et al., 2009; Govindarajan et al., 2005; Sambucetti et al., 1989). In addition, behavioral studies of ΔRG mice have revealed increased anxiety levels and mild deficits in hippocampus-dependent learning and memory, consistent with TSC-related neuropsychiatric symptoms (Chévere-Torres et al., 2011; Ehninger and Silva, 2010).

Fragile X syndrome (FXS) is caused by loss of function mutations in the RNA-binding protein, fragile X mental retardation protein (FMRP), whose normal function is to suppress translation (Ronesi and Huber, 2008). Consistent with this notion, mouse models of FXS display increased protein synthesis, enhanced mTORC1 signaling, and exaggerated metabotropic glutamate receptor-dependent long-term depression (mGluR-LTD) (Hou et al., 2006; Huber et al., 2002; Osterweil et al., 2010; Sharma et al., 2010). Based on evidence that both TSC1/2 and FMRP proteins act as negative regulators of protein synthesis and mTORC1 signaling, and the evidence that patients with TSC and FXS can both display autism-related behaviors, we hypothesized that the mutations in TSC2-GAP domain in ΔRG mice would result in similar synaptic plasticity alterations and mTORC1 dysregulation as observed in other mouse models of TSC and FXS model mice. Herein we describe experiments with the ΔRG transgenic mice that were conducted to determine whether they exhibit hippocampal synaptic plasticity phenotypes consistent with other mouse models of TSC and FXS.

Materials and methods

Animals

ΔRG transgenic mice

Generation of ΔRG mice has been described previously (Govindarajan et al., 2005). Mouse genotyping was performed by PCR using transgene- and wild-type-specific primer sets.

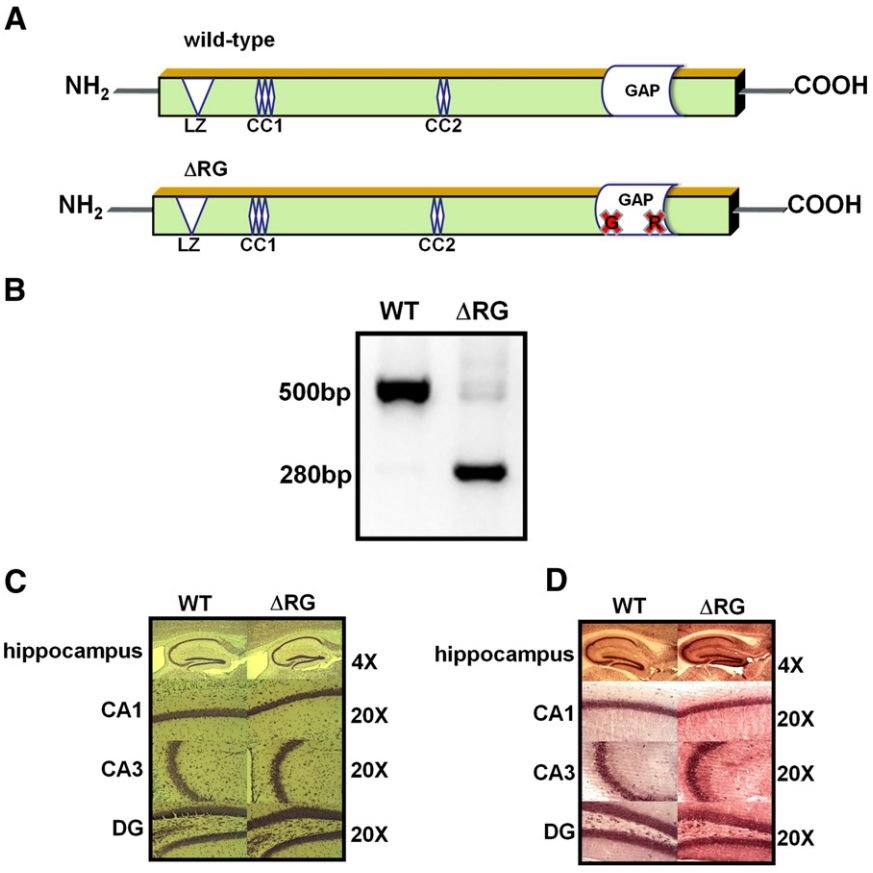

Fig. 1. Overexpression of ΔRG TSC2 protein in mouse hippocampus. (A) Schematic representation of the dominant negative TSC2 ΔRG in mice that model tuberous sclerosis. (B) PCR identification of ΔRG transgene showed a corresponding band at 280 bp. The wild-type band is detected at 500 bp. (C) Hippocampal morphology in ΔRG mice. Nissl staining of sagittal sections showed no obvious aberrant morphology. (D) Immunolocalization of TSC2 in the mouse hippocampus. Increased levels of TSC2 were observed in hippocampal areas CA1 and CA3, and the dentate gyrus (DG) of ΔRG mice compared to WT mice.