Neuroscience 172 (2011) 427–442

Pik3c3 DELETION IN PYRAMIDAL NEURONS RESULTS IN LOSS OF SYNAPSES, EXTENSIVE GLIOSIS AND PROGRESSIVE NEURODEGENERATION

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Abstract—The lipid kinase PIK3C3 (also known as VPS34) regulates multiple aspects of endo-membrane trafficking processes. PIK3C3 is widely expressed by neurons in the CNS, and its catalytic product PI3P is enriched in dendritic spines. Here we generated a line of conditional mutant mouse in which *Pik3c3* is specifically deleted in hippocampal and in small subsets of cortical pyramidal neurons using the *CaMKII-Cre* transgene. We found that *Pik3c3*-deficiency initially causes loss of dendritic spines accompanied with reactive gliosis, which is followed by progressive neuronal degeneration over a period of several months. Layers III and IV cortical neurons are more susceptible to *Pik3c3*-deletion than hippocampal neurons. Furthermore, in aged conditional *Pik3c3* mutant animals, there are extensive gliosis and severe secondary loss of wild type neurons. Our analyses show that *Pik3c3* is essential for CNS neuronal homeostasis and *Pik3c3*$^{flox/flox}$; CaMKII-Cre mouse is a useful model for studying pathological changes in progressive forebrain neurodegeneration. © 2011 Published by Elsevier Ltd on behalf of IBRO.

Key words: PIK3C3/Vps34, synapse, pyramidal neuron, neurodegeneration, reactive gliosis.

Neurons contain extensive amount of surface membranes due to their elaborate dendritic and axonal arbors. Endocytic membrane trafficking plays essential roles not only in maintaining neuronal morphological integrity, but also in cycling molecular components involved in neurotransmission and in trafficking receptors at the postsynaptic sites (De Camilli and Takei, 1996; Barry and Ziff, 2002; Malinow and Malenka, 2002; Bredt and Nicoll, 2003). Alterations in endosomal functions have been observed in a range of neurodegenerative disorders (Soreghan et al., 2003; Nixon, 2005; Bronfman et al., 2007; Lee et al., 2007).

The class III phosphoinositide 3-kinase (PIK3C3, also known as VPS34) is a member of the PI3K family lipid kinases. It specifically utilizes phosphatidylinositol as a substrate, producing the single lipid product phosphatidylinositol-3-phosphate (PI3P) (Wurmser et al., 1999). PI3P is highly enriched on early endosomes and multivesicular bodies (MVBs) and can recruit proteins containing FYVE , PX or PH motifs to these compartments (Gaullier et al., 1998; Patki et al., 1998; Gillooly et al., 2000). In invertebrate organisms and non-neuronal cells, PI3P and PIK3C3 were shown be involved in endocytic vesicular trafficking and intracellular protein sorting (Herman and Emr, 1990; Takegawa et al., 1995; Gruenberg and Stenmark, 2004; Johnson et al., 2006; Juhasz et al., 2008), as well as in the initiation of autophagy (Petiot et al., 2000; Levine and Klionsky, 2004; Zeng et al., 2006). In addition, PIK3C3 is also required for nutrient activation of mammalian targets of rapamycin (mTOR) signaling in cultured cells (Nobukuni et al., 2007; Gulati et al., 2008). Recently, a genetic study of *Pik3c3* in mammals showed that deletion of *Pik3c3* in differentiated sensory neurons causes neuronal subtype specific degenerative phenotypes, mainly due to a disruption in the endosomal but not the autophagy pathway, indicating that PIK3C3-regulated endosomal pathway is essential for sensory neuron survival and homeostasis (Zhou et al., 2010). The consequences of *Pik3c3* deletion in CNS neurons have not been characterized.

The *Pik3c3* gene is widely expressed in the developing (Zhou et al., 2010) and adult brain (http://mouse.brain-map.org/brain/Pik3c3/68498253/thumbnails.html). Moreover, PI3P is ubiquitously distributed in both dendrites and axons in cultured hippocampal neurons, with an enrichment in spines as shown by its co-localization with the postsynaptic marker PSD95 (Fig. 1A, B). To examine the roles of PIK3C3 in the maintenance of synaptic and neuronal homeostasis, we specifically deleted *Pik3c3* in mature pyramidal neurons by crossing the *CaMKII-Cre* transgenic mice with the *Pik3c3*$^{flox/flox}$ conditional null mice. Here we report our analyses of the *CaMKII-Cre; Pik3c3*$^{flox/flox}$ conditional mutant mice and show that these mice are useful models to study progressive forebrain neurodegeneration.

EXPERIMENTAL PROCEDURES

Mouse strains

Pik3c3$^{flox/flox}$ mice (described previously Zhou et al., 2010) were crossed with *CaMKII-Cre* transgenic mice (Xu et al., 2000). At 4 weeks of age, tail tissue was taken from the mice for genotyping. In all experiments, conditional mutants (*CaMKII-Cre*; *Pik3c3*$^{flox/flox}$) were compared to heterozygous *CaMKII-Cre; Pik3c3*$^{flox/+}$ littermates. At each stage/age, six pairs (conditional mutants and heterozygous controls) of mice were used for H&E, immunostaining and Golgi staining, respectively. To analyze the pattern of Cre-mediated recombination, *CaMKII-Cre* mice were crossed to the *Rosa*$^{CAG\text{-}STOP\text{-}tdTomato}$ reporter mice (hereafter shortened as *Rosa*$^{stop\text{-}tdTomato}$ mice). All experimental procedures were ap-

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Abbreviations: Cart, cocaine and amphetamine regulated transcript; DIV, days *in vitro*; Etv1, ets variant gene 1; mTOR, mammalian targets of rapamycin; PIK3C3, class III phosphoinositide 3-kinase; PtdIns3P, phosphatidylinositol-3-phosphate; Rorb, RAR-related orphan receptor beta; Wfs1, Wolfram syndrome 1 homolog.

0306-4522/11 $ - see front matter © 2011 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2010.10.035

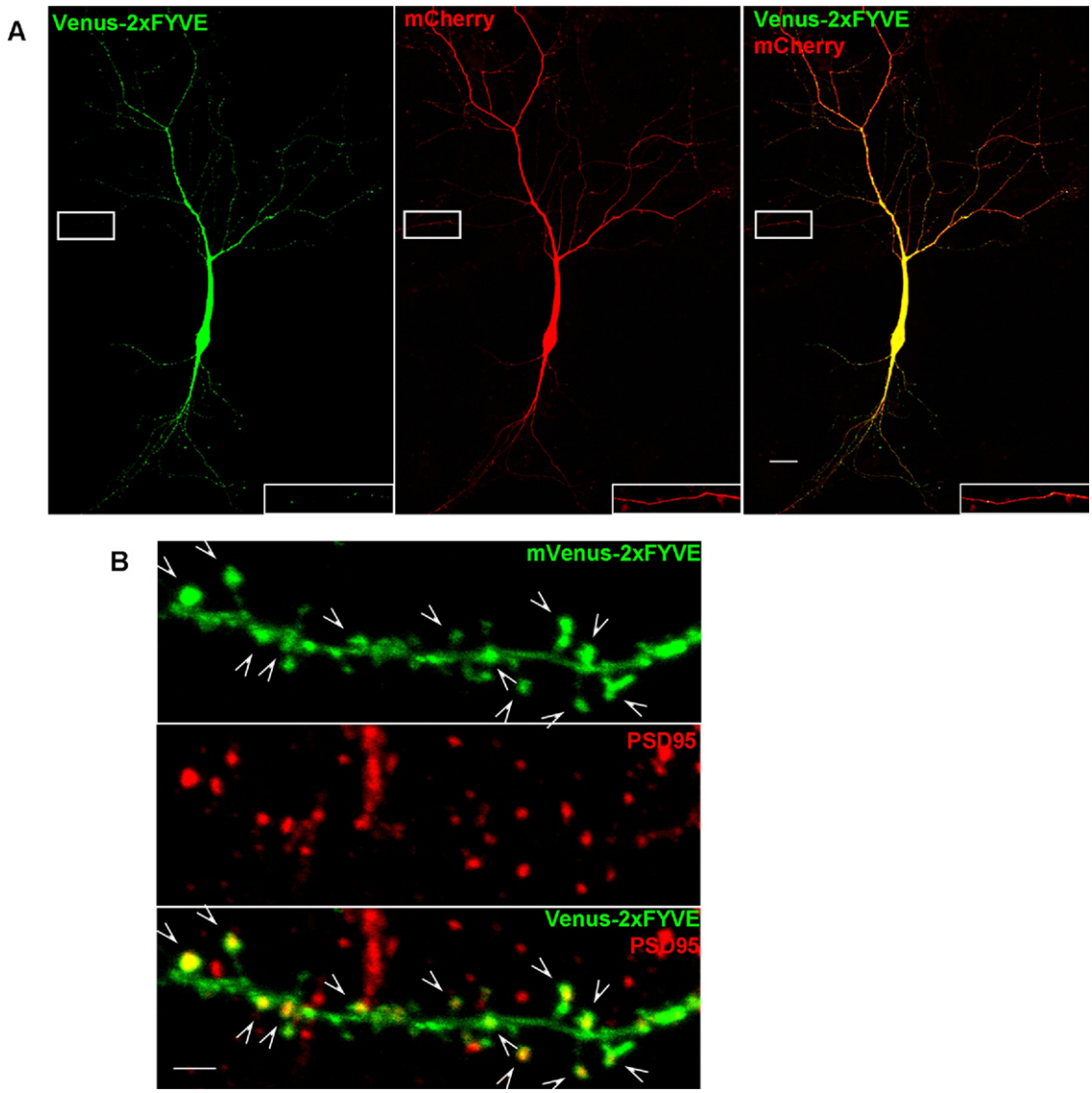

Fig. 1. Distribution of PI3P in cultured neurons revealed by mVenus-2xFYVE fusion protein. (A) Confocal images showing the intracellular distribution of PI3P in a 13 DIV hippocampal neuron cotransfected with mVenus-2xFYVE (green) and mCherry (red). The image was taken using the tiling function and multiple images were stitched together by the software automatically. The image was significantly over-exposed in order to detect the localization of PI3P in high order branches. PI3P punctae labeled by mVenus-2xFYVE are detected in both dendrites and in axon. The insets are high-power magnification of the boxed axon regions. Scale bar: 20 μm. (B) Confocal images showing partial co-localization of PI3P, as revealed by mVenus-2xFYVE (green), with postsynaptic marker PSD95 (red) in cultures neurons. Arrows indicate the co-localization. Scale bar: 2 μm.

proved by the Institutional Animal Care and Use Committee of Duke University.

Neuron culture

Dissociated hippocampal cultures were prepared from 1-d-old mice as described previously (Oh and Derkach, 2005) and transfected after 13 days *in vitro* (DIV) using a lipofectamine 2000 transfection protocol. Briefly, for a 12-well plate, 160 μl of Neurobasal per well was combined with 3.2 μl of LIPOFECTAMINE 2000™ and allowed to sit at room temperature for 5 min. Then, the solution was combined with 1 μg DNA in 160 μl of Neurobasal and allowed to sit at room temperature for 20 min (all transfection materials from Invitrogen, Rockville, MD, USA). While the solutions were complexing, the cultures were removed from the incubator and the medium was replaced with 2 ml Neurobasal. Twenty minutes later, the DNA/lipofectamine mixture was added to the culture. After 30–45 min incubation, the medium was replaced with normal culture medium. The generation of plasmid constructs expressing mVenus-2xFYVE or mCherry has been described (Zhou et al., 2010).

Tissue processing

Mice were overdosed with anesthetic and intracardially perfused with cold saline, followed by 4% paraformaldehyde. Brains were removed, post-fixed overnight. For H&E staining, fixed brain tissues were dehydrated in ethanol, embedded in paraffin. For immunocytochemistry, fixed brains were then stored in 30% sucrose at 4 °C. For Golgi staining, mice were only slightly perfused.