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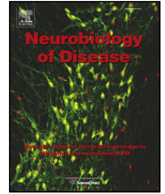

RanBP9 overexpression accelerates loss of dendritic spines in a mouse model of Alzheimer's disease

Ruizhi Wang [a], Juan Pablo Palavicini [a], Hongjie Wang [a], Panchanan Maiti [b], Elisabetta Bianchi [c], Shaohua Xu [d], B.N. Lloyd [e], Ken Dawson-Scully [e], David E. Kang [f], Madepalli K. Lakshmana [a,*]

[a] Section of Neurobiology, Torrey Pines Institute for Molecular Studies, 11350 SW Village Parkway, Port Saint Lucie, FL 34987, USA
[b] University of Tennessee Health Science Center, Department of Neurology, 415 Link Building, TN, USA
[c] Laboratory of Immuneregulation, Department of Immunology, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris, France
[d] Florida Institute of Technology, 150 West University Blvd, Melbourne, FL 32901, USA
[e] Department of Biological Sciences, Charles E. Schmidt College of Science, Florida Atlantic University, Boca Raton, FL, USA
[f] Department of Molecular Medicine, USF Health Byrd Alzheimer's Institute, 4001 E. Fletcher Ave. — MDC36, Tampa, FL 33612, USA

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ABSTRACT

We previously demonstrated that RanBP9 overexpression increased Aβ generation and amyloid plaque burden, subsequently leading to robust reductions in the levels of several synaptic proteins as well as deficits in the learning and memory skills in a mouse model of Alzheimer's disease (AD). In the present study, we found striking reduction of spinophilin-immunoreactive puncta (52%, $p < 0.001$) and spinophilin area (62.5%, $p < 0.001$) in the primary cortical neurons derived from RanBP9 transgenic mice (RanBP9-Tg) compared to wild-type (WT) neurons. Similar results were confirmed in WT cortical neurons transfected with EGFP-RanBP9. At 6-months of age, the total spine density in the cortex of RanBP9 single transgenic, APΔE9 double transgenic and APΔE9/RanBP9 triple transgenic mice was similar to WT mice. However, in the hippocampus the spine density was significantly reduced (27%, $p < 0.05$) in the triple transgenic mice compared to WT mice due to reduced number of thin spines (33%, $p < 0.05$) and mushroom spines (22%, $p < 0.05$). This suggests that RanBP9 overexpression in the APΔE9 mice accelerates loss of spines and that the hippocampus is more vulnerable. At 12-months of age, the cortex showed significant reductions in total spine density in the RanBP9 (22%, $p < 0.05$), APΔE9 (19%, $p < 0.05$) and APΔE9/RanBP9 (33%, $p < 0.01$) mice compared to WT controls due to reductions in mushroom and thin spines. Similarly, in the hippocampus the total spine density was reduced in the RanBP9 (23%, $p < 0.05$), APΔE9 (26%, $p < 0.05$) and APΔE9/RanBP9 (39%, $p < 0.01$) mice due to reductions in thin and mushroom spines. Most importantly, RanBP9 overexpression in the APΔE9 mice further exacerbated the reductions in spine density in both the cortex (14%, $p < 0.05$) and the hippocampus (16%, $p < 0.05$). Because dendritic spines are considered physical traces of memory, loss of spines due to RanBP9 provided the physical basis for the learning and memory deficits. Since RanBP9 protein levels are increased in AD brains, RanBP9 might play a crucial role in the loss of spines and synapses in AD.

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease of the elderly characterized by two neuropathological hallmarks, extracellular amyloid plaques and intraneuronal neurofibrillary tangles (Goedert and Spillantini, 2006). The progression of disease pathology is further accompanied by a marked loss of synapses. Synapse loss which best correlates with cognitive impairment (DeKosky and Scheff, 1990; Scheff et al., 1990, 2007; Terry et al., 1991) has been reported as an early event in the pathogenesis of AD. In fact, dendritic spines which are considered structural correlates of learning and memory (Alvarez and Sabatini, 2007; Nimchinsky et al., 2002) have been reported to be substantially reduced in AD brains (Fiala et al., 2002; Knobloch and Mansuy, 2008; Merino-Serrais et al., 2013). Since dendritic spines represent the major postsynaptic elements of excitatory synapses in the brain and are fundamental to long-term potentiation (LTP) and long-term depression (LTD), which are considered the predominant cellular mechanisms that underlie learning and memory (Cooke and

Abbreviations: AD, Alzheimer's disease; APP, Amyloid precursor protein; LRP, low-density lipoprotein receptor-related protein; LTD, long-term depression; LTP, long-term potentiation; MAP2, Microtubule associated protein 2; NP40, Nonidet-P40; PFA, Paraformaldehyde; PS1, Presenilin 1; PVDF, Polyvinylidene fluoride; RanBP9, Ran-binding protein 9.

* Corresponding author at: Torrey Pines Institute for Molecular Studies, 11350 SW Village Parkway, Port Saint Lucie, FL 34987-2352, USA. Fax: +1 772 345 3649.
E-mail address: mlakshmana@tpims.org (M.K. Lakshmana).

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Bliss, 2006), understanding the mechanisms by which spine loss occurs is important to unravel the pathogenesis of AD.

While there are obvious limitations in studying the spine loss directly in human brains, transgenic mouse models of AD provide great opportunity to study various aspects of spine loss including spatiotemporal correlations between the pattern of spine loss and learning and memory skills. Thus, several transgenic mouse models of AD overexpressing amyloid precursor protein (APP) and/or presenilin 1 (PS1), recapitulate loss of spines (Lanz et al., 2003; Moolman et al., 2004; Spires et al., 2005; Tsai et al., 2004). More recent studies have confirmed loss of spines in the APP/PS1 mice (Meng et al., 2013), J20 mice expressing both the Swedish (K670N/M671L) and Indiana (V717F) mutations (Pozueta et al., 2013), Tg2576 mice expressing APP with the Swedish mutation (Perez-Cruz et al., 2011), PS1 transgenic mice (Auffret et al., 2009) as well as the SAMP8 mouse model of aging (del Valle et al., 2012). The overexpression of human tau in transgenic mouse model also reduces spine density (Rocher et al., 2013). Thus, rare familial AD (FAD)-associated gene mutations within APP, PS1 as well as tau hyperphosphorylation have been shown to induce spine loss and associated memory deficits. Interestingly, in addition to FAD associated genes, those genes that increase the risk of developing AD by their genetic association also contributes to loss of spines. The APOE4 allele is the strongest risk factor identified so far for developing late-onset AD (LOAD). A recent study provided compelling evidence that the APOE4 allele significantly reduced dendritic spine density which was well-correlated with learning and memory deficits (Rodriguez et al., 2013). However, it is not clear whether other genes that increase the risk of AD or the genes that are associated with progression of AD also adversely affect spine density.

The Ran-binding protein 9 (RanBP9) was first identified as a 55 kDa protein (Nakamura et al., 1998), but later studies by the same group revealed that the full-length RanBP9 is a 90 kDa protein (Nishitani et al., 2001). RanBP9 is ubiquitously expressed in different tissues and cell lines and is highly conserved in different organisms (Rao et al., 2002; Wang et al., 2002). RanBP9 is a multidomain protein that functions as a scaffolding protein by assembling multiprotein complexes in different subcellular regions, thereby mediates diverse cellular functions (Murrin and Talbot, 2007; Suresh et al., 2012). Recently, RanBP9 was found to be within the clusters of RNA transcript pairs associated with markers of AD progression (Arefin et al., 2012), suggesting that RanBP9 might contribute to the pathogenesis of AD. In fact, even before this discovery, we showed for the first time that RanBP9 increased Aβ generation by 4-fold in a variety of cell cultures (Lakshmana et al., 2010), primary neurons (Lakshmana et al., 2009) as well as mouse brains (Lakshmana et al., 2012), consequently leading to increased amyloid plaque burden (Lakshmana et al., 2012). Because RanBP9 protein levels are increased in J20 (Woo et al., 2012) and APΔE9 mice (Wang et al., 2013) as well as in the AD brains (Lakshmana et al., 2010; Palavicini et al., 2013a), RanBP9 is expected to positively contribute to the increased Aβ generation and to the associated synaptic and behavioral deficits seen in AD patients and in mouse models of AD. In line with these predictions, we recently demonstrated that RanBP9 overexpression in the APΔE9 mice led to learning and memory deficits in both the T maze (Palavicini et al., 2013a) and Barnes maze paradigms (Woo et al., 2012). These deficits appear to be due to RanBP9-mediated synaptic damage as reflected by reduced levels of synaptic proteins in the APΔE9 mouse brains (Lakshmana et al., 2012; Palavicini et al., 2013a,2013b). Most importantly, we confirmed the inverse relationships between the protein levels of spinophilin, a marker of dendritic spines and RanBP9 levels in the synaptosomes derived from both mouse brains and AD brains (Palavicini et al., 2013a). These pieces of evidence taken together imply that RanBP9 might play a primary role in the loss of synapses in AD.

The primary objective of the present study was to examine whether RanBP9 overexpression in APΔE9 mice leads to alterations in dendritic spines. Remarkably, RanBP9 overexpression in APΔE9 mice accelerated loss of spines already at 6-months of age. Thus, the loss of spines observed in the present study provides the physical basis for the previously observed synaptic and behavioral deficits due to RanBP9 overexpression in the APΔE9 mouse model of AD.

Material and methods

Mice

All animal experiments were carried out based on the ARRIVE guidelines and in strict accordance with the National Institutes of Health's 'Guide for the Care and Use of Animals' and as approved by the Torrey Pines Institute's Animal Care and Use Committee (IACUC). Generation of RanBP9-Tg mice has been described previously (Lakshmana et al., 2012). The RanBP9 specific primers used in the polymerase chain reaction (PCR) are as follows. The forward primer is 5′-gcc acg cat cca ata cca g-3′, and the reverse primer is 5-tgc ctg gat ttt ggt tct c-3′. Positive mice were then backcrossed with native C57Bl/6 mice and the colonies were expanded. RanBP9-Tg line 629 was used to breed with B6.Cg-Tg, APPswe, PSEN1ΔE9 (APΔE9) mice for generating triple transgenic mice (APΔE9/RanBP9). We obtained APΔE9 mice from Jackson Labs (Bar Harbor, Maine, USA). These double transgenic mice express a chimeric mouse/human APP (Mo/HuAPP695swe) driven by prion promoter and a mutant human presenilin 1 (PS1-ΔE9) also driven by the prion promoter for neuronal expression of transgenes. These APΔE9 transgenic mice were generated by co-injection of APP695swe and PS1-ΔE9 encoding vectors controlled by their own mouse prion promoter element. These mice were backcrossed to maintain them in the C57Bl/6 background, expanded and genotyped to confirm the transgene using the following primers. The forward primer is 5′-gac tga cca ctc gac cag gtt ctg-3′ and the reverse primer is 5-ctt gta agt tgg att ctc ata tcc g-3′. The mice were fed with ad libitum food and water all the time. The food is the irradiated global rodent chow from Harlan. The mice were maintained in a 12-hour light/dark cycle at a temperature of 21–23 °C and a humidity of 55 ± 10. After weaning, mice were kept in home cages comprising single sex, single genotype and groups of only 5 mice per cage. All of the mice lived in an enriched environment with increased amounts of bedding and nesting materials.

Primary neuronal cultures

To prepare cortical primary neuronal cultures, cortices from both the hemispheres were separated and freed from meninges under a dissection microscope from newborn (P0) pups of RanBP9 transgenic mice overexpressing flag-tagged RanBP9 (RanBP9-Tg) or from wild-type (WT) mice. The cortical tissue was washed 3× with Ca^{2+}/Mg^{2+}-free Hanks' balanced salt solution containing penicillin/streptomycin. The tissues were dissociated in 0.27% trypsin (in 10% Dulbecco's modified Eagle's medium/Hanks' balanced salt solution) by incubating at 37 °C for 30 min. Neurons were collected by centrifugation and re-suspended in 10% Ham's F-12 medium (cat # 10-080-CV, Media Tech, Pittsburgh, PA, USA) containing penicillin/streptomycin. The neurons were further dissociated by triturating 20 times with a Pasteur pipette and passed through a cell strainer. After centrifugation, the neurons were re-suspended in neurobasal medium containing 2% B-27 supplement (cat # 1-7504-044, Life Technologies, Grand Island, NY, USA), glutamine (cat# 25030-081, Life Technologies), pyruvate (cat # 11360, Life Technologies), and penicillin/streptomycin (50 units/ml penicillin, 50 μg/ml streptomycin, cat # 30-002-C1, Media Tech) and plated on to a sterile coverslip in a 6-well plate. The coverslips (GG-18-PDL, Neuvitro, Germany) used were especially made for primary neurons, coated with Poly-D-Lysine (PDL). Half of the growth medium was changed twice weekly.

Sholl analysis

To understand the influence of RanBP9 on dendritic intersections, primary cortical neurons from WT and RanBP9-Tg mice were immunostained with MAP2 (1:150 dilution) and dendritic intersections were

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