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Kalirin-7 prevents dendritic spine dysgenesis induced by amyloid beta-derived oligomers

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Abstract

Synapse degeneration and dendritic spine dysgenesis are believed to be crucial early steps in Alzheimer's disease (AD), and correlate with cognitive deficits in AD patients. Soluble amyloid beta (Aβ)-derived oligomers, also termed Aβ-derived diffusible ligands (ADDLs), accumulate in the brain of AD patients and play a crucial role in AD pathogenesis. ADDLs bind to mature hippocampal neurons, induce structural changes in dendritic spines and contribute to neuronal death. However, mechanisms underlying structural and toxic effects are not fully understood. Here we report that ADDLs bind to cultured mature cortical pyramidal neurons and induce spine dysgenesis. ADDL treatment induced the rapid depletion of kalirin-7, a brain-specific guanine-nucleotide exchange factor for the small GTPase Rac1, from spines. Kalirin-7 is key regulator of dendritic spine morphogenesis and maintenance in forebrain pyramidal neurons and here we show that overexpression of kalirin-7 prevents ADDL-induced spine degeneration. Taken together, our results suggest that kalirin-7 may play a role in the early events leading to synapse degeneration, and its pharmacological activation may prevent or delay synapse pathology in AD.

Keywords

Alzheimer's disease; guanine nucleotide exchange factors; synapse; kalirin; Rac1

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Author contributions

Study concept and design: ZX, MC, PL, WK, PP. Data acquisition: ZX, MC. Data analysis and interpretation: LS and PP. Drafting the manuscript: TR. Critical revision of the manuscript: LS and PP. Obtained funding: PP. Administrative, technical and material support: WL and PP. Study supervision: PP.

Conflict of interest

None of the authors have completing financial interests in relation to the work described.

Data accessibility

Data associated with the manuscript will be available upon request.

1. Introduction

Fibrillar amyloid plaques are among the hallmark neuropathological features of Alzheimer's disease (AD), and are composed of aggregates of Aβ, a proteolytic cleavage product of amyloid precursor protein (Hardy and Selkoe 2002). It is well established that amyloid plaques induce synaptic degeneration; however, much evidence indicates that the early cognitive deficits of AD result from synaptic dysfunction due to the accumulation of soluble oligomeric Aβ-derived diffusible ligands (ADDLs), which are elevated in the brain and cerebrospinal fluid of AD patients (Georganopoulou *et al.* 2005, Selkoe and Hardy 2016). Soluble Aβ inhibits long-term potentiation (Shankar *et al.* 2008, Spires-Jones and Knafo 2012, Wang *et al.* 2002) and results in a reduction in forebrain dendritic spine density (Calabrese *et al.* 2007, Lacor *et al.* 2007), and the cognitive and spine deficits of AD mouse models ensue at a time of elevated soluble Aβ levels (Jacobsen *et al.* 2006).

Synapse degeneration and dendritic spine dysgenesis are crucial early steps in AD (Selkoe 2002) and in animal models of AD (Almeida *et al.* 2005, Jacobsen *et al.* 2006, Lanz *et al.* 2003, Moolman *et al.* 2004, Spires *et al.* 2005), and correlate well with cognitive deficits in AD patients (Counts *et al.* 2006, Ji *et al.* 2003). Spine structural plasticity is ultimately mediated by a change in the ratio of filamentous F-actin and globular G-actin, with F-actin being enriched in more stable, mature spines. Synaptosomes from both AD patient brain samples and AD mouse model brains display decreased levels of F-actin; furthermore, exposure of cortical neurons to $A\beta_{1-42}$ is sufficient to cause a similar reduction in F-actin in spines (Kommaddi *et al.* 2018). Dendritic spine markers spinophilin and PSD-95 are also reduced in the cerebral cortex of AD patients (Gylys *et al.* 2004, Mi *et al.* 2017). Nevertheless, the signaling cascades responsible for these changes remain poorly understood.

ADDLs specifically bind to excitatory neurons and have been shown to colocalize with PSD-95, but not the inhibitory synapse marker gephyrin (Lacor *et al.* 2004; Klein 2006; Lacor *et al.* 2007; Renner *et al.* 2010). Many studies have sought to understand how ADDLs target excitatory neurons and the mechanisms by which they induce dendritic spine atrophy (discussed in Penzes and Vanleeuwen 2011; Wilcox *et al.* 2011; Koleske 2013). ADDLs can interact directly with postsynaptic NMDA and AMPA glutamate receptors, and lead to the removal of these receptors from the synapse (Knobloch and Mansuy 2008). In addition to glutamate receptors, other signaling molecules important in spine morphogenesis/plasticity and implicated in memory formation are down-regulated by soluble amyloid, including the receptor tyrosine kinase EphB2, and the F-actin stabilizing protein drebrin (Lacor *et al.* 2007, Roselli *et al.* 2005, Zhao *et al.* 2006). The Rho family of small GTPases are also hypothesized to contribute to dendrite spine shrinkage in AD, however; the exact mechanisms by which Rho GTPase is involved in ADDL-induced atrophy is not fully understood (Pozueta *et al.* 2013).

We have previously identified the protein kalirin as a brain-specific guanine-nucleotide exchange factor, which activates the Rho family GTPase Rac1 (Penzes *et al.* 2003, Penzes *et al.* 2001a). Kalirin is expressed most highly in the hippocampus and cortex and is only present at low levels outside of the brain (Ma *et al.* 2001). Its most abundant form, kalirin-7,

is concentrated in dendritic spines and interacts with the major postsynaptic scaffolding protein PSD-95 (Penzes *et al.* 2001b). In pyramidal neurons, kalirin-7 controls dendritic spine formation, maintenance, and remodeling via its promotion of actin polymerization (Penzes *et al.* 2003, Penzes *et al.* 2001a). Kalirin-7 also interacts with several postsynaptic proteins that are affected in AD and which contribute to AD pathology, including p21-activated kinase (PAK) (Penzes *et al.* 2003), inducible nitric oxide synthase (Ratovitski *et al.* 1999), X11α (Penzes *et al.* 2001b), and GluA1 (Xie *et al.* 2007).

Importantly, KALRN has been identified as the most consistently underexpressed gene in the hippocampus and frontal cortices of individuals with AD (Youn *et al.* 2007; Murray *et al.* 2012). Aβ treatment of cultured hippocampal neurons reduces kalirin-7 mRNA expression, and overexpression of XBP1, a transcription factor which promotes kalirin expression, has been shown to rescue spine deficits in the hippocampus of a mouse model of AD. This effect was prevented by concomitant knockdown of kalirin-7 (Cisse *et al.* 2017a,b).

Given that the selective memory deficits and spine dysfunction present in kalirin-knockout (KO) animals closely parallel that of human AD and early stages in AD animal models (Cahill *et al.* 2009; Xie *et al.* 2010; Xie *et al.* 2011; Vanleeuwen and Penzes 2012), we aimed to determine whether kalirin-7 is involved in ADDL-induced spine dysfunction. We report that kalirin-7 localization is altered ADDL-treated cortical neurons and our data suggest that enhancing kalirin-7 may be a mechanism to slow ADDL-induced spine atrophy.

2. Materials and Methods

2.1 Antibodies and reagents

The plasmid encoding myc-kalirin-7 and a rabbit polyclonal antibody against kalirin-7 have been described previously (Penzes *et al.* 2000; 1:500), as has a mouse monoclonal antibody against ADDLs (Lambert *et al.* 2007; 1:2000). The pEGFP-N2 plasmid was purchased from Clontech (Mountain View, CA). The following antibodies were used: chicken anti-GFP (RRID:AB_300798; Abcam; Cambridge, MA; 1:1000); mouse anti-myc (RRID:AB_627268; Santa Cruz Biotechnology; Dallas, TX; 1:1000); Alexa Fluor 488 goat anti-chicken IgY (RRID:AB_2534096; 1:1000), Alexa Fluor 488 goat anti-mouse IgG (RRID:AB_2534084; 1:1000), Alexa Fluor 568 goat anti-mouse IgG (RRID:AB_2534072; 1:1000), and Alexa Fluor 568 goat anti-rabbit IgG (RRID:AB_10563566; 1:1000) (all from Invitrogen; Carlsbad, CA).

2.2 Neuronal culture and transfections

Medium and high density cortical neuron cultures were prepared from Sprague-Dawley rat (RRID:MGI:5651135) E18 embryos as described previously (Xie *et al.* 2007). Animals were singly housed on a 12:12 light-dark cycle, given ad libitum access to food and water, and euthanized via CO_2. Neurons were plated onto coverslips coated with poly-D-lysine (0.2 mg/ml) (Sigma; St. Louis, MO), in plating media (feeding media plus 5% fetal calf serum). After 1 h, the media was changed to feeding media (Neurobasal media supplemented with B27 (Invitrogen) and 0.5 mM glutamine (Invitrogen)). 200 μM D,L-amino-phosphonovalerate (D,L-APV) (Ascent Scientific; Cambridge, MA) was added to the media

4 days later. Neurons were transfected at DIV 26 using Lipofectamine 2000 following the manufacturer's recommendations. Briefly, between 2–5 μg of each DNA was mixed with 4 μl of Lipofectamine 2000 in 100 μl feeding media; transfections were allowed to carry on for 2 days.

2.3 Neuronal treatments

ADDLs were generated as described previously (Lambert *et al.* 2001; Klein 2002). Neurons were treated with ADDLs or vehicle for either 2 or 16 h as indicated in the Results. Alternatively, neurons were treated with 1 mM activated sodium vanadate (New England BioLabs, Ipswich, MA) or vehicle for 2 h. Following incubation for the given times, cells were immediately fixed and immunostained.

2.4 Immunostaining

Neurons were fixed in either 4% formaldehyde, 4% sucrose in PBS for 10 min, or in 4% formaldehyde, 4% sucrose in PBS followed by 10 min of fixation with methanol pre-chilled to −20°C. Neurons were then permeabilized and blocked simultaneously in PBS containing 2% normal goat serum and 0.2% Triton-X-100 for 1 h at room temperature. Primary antibodies were added in PBS containing 2% normal goat serum overnight at 4 °C, followed by three 10 min washes in PBS. Secondary antibodies were incubated for 1 h at room temperature, also in 2% normal goat serum in PBS. Three further washes in PBS were performed before coverslips were mounted with ProLong Antifade Reagent (Invitrogen, Carlsbad, CA). In the case of co-transfections with eGFP-N2 and kalirin-7-myc plasmids, neurons were stained with both anti-GFP and anti-myc antibodies.

2.5 Quantitative analysis of spine density and dendritic expression of kalirin-7

Confocal images of single- and double-stained neurons were obtained with a Zeiss LSM5 Pascal confocal microscope. To determine the lateral resolution of our confocal microscope, we imaged fluorescent microspheres of known diameters, mounted in ProLong antifade solution, using the 63× oil-immersion objective (NA = 1.4). An anti-GFP antibody was used to circumvent potential unevenness of GFP diffusion in spines. Images of neurons were taken using the 63X oil-immersion objective (NA = 1.4) as a z-series of 3–8 images, averaged 4 times, at 0.37 μm intervals. The acquisition parameters were kept the same for all scans. Two-dimensional maximum projection reconstructions of images, morphometric analysis and quantification were done using MetaMorph software (Molecular Devices, Sunnyvale, California). Only healthy excitatory neurons with homogenous GFP staining, and intact secondary and tertiary apical and basilar dendrites were imaged. If dendritic blebbing was detected, neurons were excluded. Co-transfection with kalirin-7 and GFP plasmids was confirmed before image acquisition. Excitatory neurons were identified by their prominent apical dendrite and numerous thinner basilar dendrites. Cultures that were directly compared were stained simultaneously and imaged with the same acquisition parameters. Kalirin-7 immunoreactivity expression in dendrites and spines was determined using ImageJ (NIH, Bethesda, Maryland).

To examine the morphologies of dendritic spines, individual spines on dendrites were manually traced, and each spine was measured by MetaMorph. Based upon morphological

measurements, spines were classified as thin, stubby, mushroom or filopodia (Risher *et al.*, 2014). All data was collected and analyzed by an experimenter who was blind to the experimental treatment.

2.6 Statistical analysis

Statistical analyses (Student's unpaired t-test, one-way ANOVA) were performed in Excel and SPSS. Tukey-b *post hoc* analysis was used for multiple comparisons.

3. Results

3. 1. ADDLs bind to dendrites and spines of cortical neurons

Studies have shown that binding of ADDLs composed of soluble oligomeric $A\beta_{1-42}$ to the spines of hippocampal neurons may be crucial for induction of spine dysgenesis and cognitive loss in AD (Lacor *et al.* 2007). Further, long-term ADDL treatment induces death of human cortical neurons (Deshpande *et al.* 2006). Here, we investigated the ability of ADDLs to bind to dendrites and dendritic spines of cortical pyramidal neurons. We transfected mature (DIV 28) cultured rat cortical neurons with GFP and treated them with 500 nM ADDLs for 2 hours, then immunostained with antibodies against ADDLs and GFP and imaged using confocal microscopy (Fig.1a-e). We found that approximately 75% of dendritic spines were bound by ADDLs (Fig. 1c, arrowheads in 1d); however, binding also occurred along the dendrite (Fig. 1d). This pattern of binding is comparable to that which has been observed in cultured hippocampal pyramidal neurons (Lacor *et al.* 2007).

3.2 ADDLs induce rapid changes in kalirin-7 localization

Kalirin-7 is expressed at lower levels in both AD patient hippocampi and frontal cortices as well as in the brains of AD model mice (Youn *et al.* 2007; Murray *et al.* 2012; Cisse *et al.* 2017b,), and siRNA-mediated knockdown of kalirin-7 in cortical neurons leads to reductions in spine area (Xie *et al.* 2007). Further, kalirin-7 has been shown to be vital for spine function and maintenance, and its expression is altered in multiple neuropsychiatric disorders (Penzes and Jones 2008; Penzes and Remmers 2012; Remmers *et al.* 2014). For these reasons, we examined the effect of exogenous ADDLs on the localization of endogenous kalirin-7 in dendritic spines (Fig. 2a,b). ADDL did not significantly reduce total kalirin-7 immunoreactivity [$t_{(29)}$=1.67, p=0.11] (Fig. 2c); however, it reduced the ratio of kalirin-7 in spines to dendrites [$t_{(28)}$=4.17, p<0.001] (Fig. 2c). These data indicate that ADDLs rapidly alter kalirin-7 localization, suggesting that modification of kalirin-7 localization is an early event that potentially contributes to oligomeric amyloid pathogenesis.

3.3 Kalirin-7 overexpression prevents dendritic spine dysgenesis caused by ADDLs

Treatment of both dissociated and organotypic hippocampal cultures with Aβ oligomers alters dendritic spines (Lacor *et al.* 2007; Smith *et al.* 2009) and transcriptional activation of kalirin-7 has been shown to reverse the reductions in spine density seen in the hippocampus of AD transgenic mice (Cisse *et al.* 2017b). Further, we observed that ADDLs bind to cortical neurons in a similar pattern as they do to hippocampal neurons (Lacor *et al.* 2007). Thus, we first evaluated whether ADDL treatment would induce spine dysgenesis in cortical neurons and then assessed if overexpression of kalirin-7 would prevent any ADDL-induced

spine dysgenesis. We transfected cortical neurons with a kalirin-7 expressing plasmid or GFP plasmid for two days before treating neurons with ADDLs for 16 hours (Fig. 3a,b). We then analyzed dendritic spine density and morphology. ADDLs increases the average length of all spines but overexpression of kalirin-7 occludes this effect [$F_{(2,35)}$ =9.06, p<0.001] (Fig. 3c). We also observed that ADDL treatment reduces density of dendritic spines, but in this case, overexpression of kalirin-7 only partially blocked spine loss [$F_{(2,34)}$=4.85, p=0.01] (Fig. 3d). We next classified dendritic spines as mushroom, thin, stubby and filopodia. ADDL treatment severely reduced the percentage of mushroom spines, but kalirin-7 overexpression prevented mushroom spine loss [$F_{(2,35)}$=14.62, p<0.0001], (Fig. 3e). Filopodia accounted for a greater percentage, approximately 38%, of total dendritic spines in ADDL-treated neurons, compared to 25% of spines in vehicle-treated neurons. Notably, kalirin-7 overexpression also occluded this increase [$F_{(2,35)}$=8.19, p=0.001], (Fig. 3f). ADDL-treatment did not alter the percentage of thin [$F_{(2,35)}$=0.08, p=0.92] (Fig. 3g) or stubby spines [$F_{(2,35)}$=0.087, p=0.43] (Fig. 3h). Filopodia are less stable than mature, bulbous, mushroom-shaped spines that most commonly host synapses (Yoshihara *et al.* 2009). As such, these sorts of changes in morphology following ADDL treatment would be expected to alter circuit properties in an intact brain and may underlie the cognitive deficits in AD. Taken together our data indicate not only that that ADDLs promote an immature spine phenotype and destabilize mature spines, but also that increasing kalirin-7 levels prevents ADDL-induced spine atrophy.

4. Discussion

Here we show that ADDLs bind to dendritic spines and dendrites of cultured mature cortical pyramidal neurons. ADDL treatment alters endogenous kalirin-7 localization, an effect that is associated with loss of dendritic spine density and an increase in dendritic spine length. This immature spine phenotype is prevented by overexpression of kalirin-7, thus a possible mechanism by which ADDLs induce spine morphological changes may be the removal of kalirin-7 from spines. Taken together, these data suggest that kalirin-7 may play a role in AD neuropathology.

There is an urgent need for the development of effective treatments that prevent, slow, or revert memory deficits. Aberrant synaptic signal transduction, as well as abnormal synaptic structure and function, are thought to be crucial contributors to the pathophysiology of AD (Selkoe 2002). However, the mechanisms linking structural and functional alterations in synapses in patient brains with the loss of their cognitive abilities are not fully understood. Consequently, the development of effective treatments for AD will be significantly facilitated by a better understanding of the mechanisms underlying synaptic pathology and by the identification of novel drug targets based on the links between synaptic pathology and cognitive deficits (Coleman *et al.* 2004). Based on our data, kalirin-7 may be one such target.

We have previously shown that kalirin-KO mice express altered neuronal morphology and structural changes as well as behavioral abnormalities that resemble certain features of AD. For example, dendritic spine density on pyramidal neurons in kalirin-KO animals is unchanged in juvenile animals but is significantly reduced in the cortex by three months of age and in the hippocampus by one year (Cahill *et al.* 2009; Vanleeuwen and Penzes 2012).

Kalirin-KO mice also exhibit a reduction in hippocampal cell number and area and a thinning of the frontal cortex (Xie *et al.* 2010, 2011). These morphological and macroanatomical changes correlate with deficits in cognitive tasks known to engage the cortex and hippocampus, including working memory as assessed by the Morris water maze and Y-maze; contextual and cued fear conditioning; and social recognition (Cahill *et al.* 2009; Xie *et al.* 2011; Vanleeuwen and Penzes 2012).

Biochemically, kalirin-7 functions in a pathway in which multiple members have been linked to AD. We have previously demonstrated that Fyn phosphorylates kalirin-7 in heterologous cultures and induces an increase in Rac1 activation (Cahill *et al.* 2011). Although total Fyn levels are unchanged in the cortices of AD patients, the ratio of Fyn levels in patients' neuronal cell bodies to that in synapses is increased (Ho *et al.* 2005). Fyn is activated downstream of Aβ binding to prion protein (PrP^C), leading to its phosphorylation of multiple synaptic proteins, and hippocampal cultures from mice lacking either PrP^C or Fyn were resistant to the loss of spines induced by Aβ (Um *et al.* 2012).

Kalirin-7 is also phosphorylated by EphB2, which leads to a clustering of kalirin-7 in the synapse and is required for ephrinB-induced spine formation (Penzes *et al.* 2003). Aβ oligomers have been shown to bind EphB2 in cultured hippocampal neurons, leading to its degradation (Lacor *et al.* 2007; Shi *et al.* 2016). Conversely, overexpression of EphB2 in the hippocampus of AD model mice is capable of preventing the loss of NMDA receptors and cognitive deficits caused by excess $A\beta_{1-42}$ exposure (Cisse *et al.* 2011; Miyamoto *et al.* 2016; Hu *et al.* 2017). Kalirin-7 directly interfaces with NMDA receptors, and this interaction is critical for synaptic plasticity (Kiraly *et al.* 2011). ADDLs have been shown to bind NDMA receptors (De Felice *et al.* 2007), highlighting another possible mechanism by which kalirin-7 may contribute to AD neuropathology.

Kalirin-7 stimulates Rac1 which activates PAK, which then phosphorylates numerous substrates involved in F-actin polymerization and stabilization (Penzes *et al.* 2003). Both AD patient brains and AD mouse model brains, as well as cultured neurons, display abnormal PAK activation. Specifically, the ratio of phospho-PAK in membrane fractions versus cytosolic fractions is increased, and the translocation of phospho-PAK to the membrane was coupled with a change in co-localization with Rac1 (Zhao *et al.* 2006; Ma *et al.* 2008).

Here, we report that ADDL treatment alters kalirin-7 localization in dendrites and dendritic spines. As the total kalirin-7 levels remain unchanged, one possible explanation is that kalirin-7 is moving from the dendritic spines to the dendrite. We also observe that ADDL treatment induces an immature spine phenotype characterized by a loss of mushroom spines and an increase in filopodial-like spines. Given that kalirin-7 co-localizes with PSD-95 and stabilizes spines, it is not surprising that kalirin-7 is not as abundant in the immature filopodial spines that are less likely to host synapses than mature mushroom-shaped spines. Also, of note, we observe a decrease in dendritic spines density with ADDL treatment. Despite this spine loss, we do not see an overall reduction in kalirin-7 levels, suggesting that as the spines shrink, kalirin-7 accumulates in the dendrite. Additional studies, such as live imaging, are needed to determine kalirin-7 mobility during dendritic spine formation and elimination.

Based upon the literature discussed above and the data presented here, we developed a model by which ADDLs induce dendritic spine atrophy and contribute to AD pathology by disrupting kalirin-7 (Fig. 4). In this model, ADDLs alter the localization of kalirin-7 in dendrites and dendritic spines by stimulating kalirin-7 binding partners, such as EphB2 and NMDA receptors. The abnormal localization of kalirin-7 disrupts Rac1 and PAK signaling and contributes to spine atrophy. With elevated kalirin-7 levels, in the case of overexpression, kalirin-7 mediated spine stabilization and downstream signaling is unaffected despite ADDL exposure. Thus, we postulate that ADDLs may cause spine atrophy by interfering with kalirin-7 localization and signaling, although live imaging studies are needed to directly test this hypothesis.

Taken together, our study fills an important gap in knowledge about a key component of the pathway linking amyloid production with synapse dysgenesis in AD. Several publications cited in this article have reported that upstream regulators of kalirin-7, including EphB2 and NMDA receptors bind ADDLs, while a downstream target of kalirin-7, PAK, is essential for synapse dysfunction in AD. Moreover, postmortem studies have shown that kalirin-7 is downregulated in brains of patients with AD, but the cellular impact of these findings was not clear. Here we reveal the cellular consequence of altered levels of kalirin-7 in the context of a model of AD. These findings could pave the way to the development of new therapeutic approaches to rescue spine dysgenesis.

Conclusions

In our present study, we have employed a cellular model of AD-relevant soluble amyloid pathology, and showed that aberrant kalirin-7 regulation is a consequence of this pathology and contributes to dendritic spine atrophy, thereby implicating kalirin-7 as another potential therapeutic target for AD. The data presented here indicate that ADDLs 1) bind to both dendrites and dendritic spines; 2) rapidly alter endogenous kalirin-7 localization; 3) induce an immature dendritic spine phenotype characterized by a loss of mature mushroom spines and an increase in dendritic spine length. Notably, these effects are prevented by overexpression of kalirin-7. Thus, we propose that ADDL exposure impairs kalirin-7 localization contributing to dendritic spine destabilization, identifying a novel mechanism to counter neuronal phenotypes associated with AD.

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Abbreviations used:

AD	Alzheimer’s disease
Aβ	amyloid beta
ADDL	amyloid beta-derived diffusible ligand
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

D,L-APV	D,L-amino-phosphonovalerate
GFP	green fluorescent protein
NMDA	N-methyl-D-aspartate
PAK	p21-activated kinase
PBS	phosphate-buffered saline
PrPC	prion protein
PSD-95	postsynaptic density protein 95

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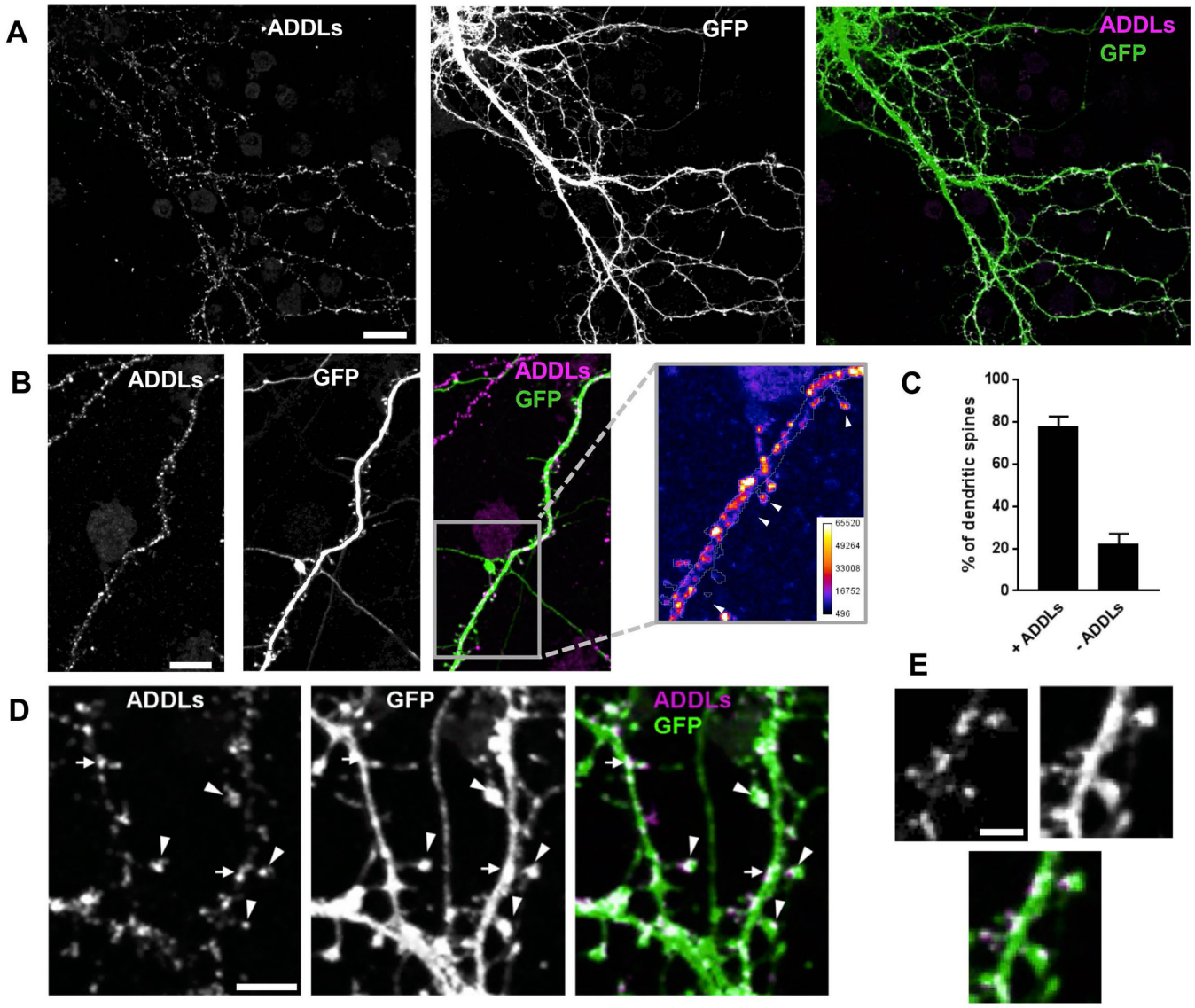

Fig. 1. ADDLs bind to cortical neurons.

Cultured rat cortical neurons (DIV 28) were treated with 500 nM ADDLs for 2 h, then immunostained with antibodies against ADDLs and GFP. (a-b) ADDLs bind to both the dendrite and the dendritic spine. Inset: heat map of ADDL mean intensity. Arrow heads indicate spines. Scale bar = 20 μm (a) and 10 μm (b). (c) Quantification of ADDL + spines indicates that approximately 75% of spines were bound by ADDL. (d-e) High magnification images further illustrate that ADDLs bind to the head of dendritic spines (arrowheads) and along the dendrite (arrows). Data were collected from 8 dendrite segments from 4 neurons. Scale bar = 5 μm.

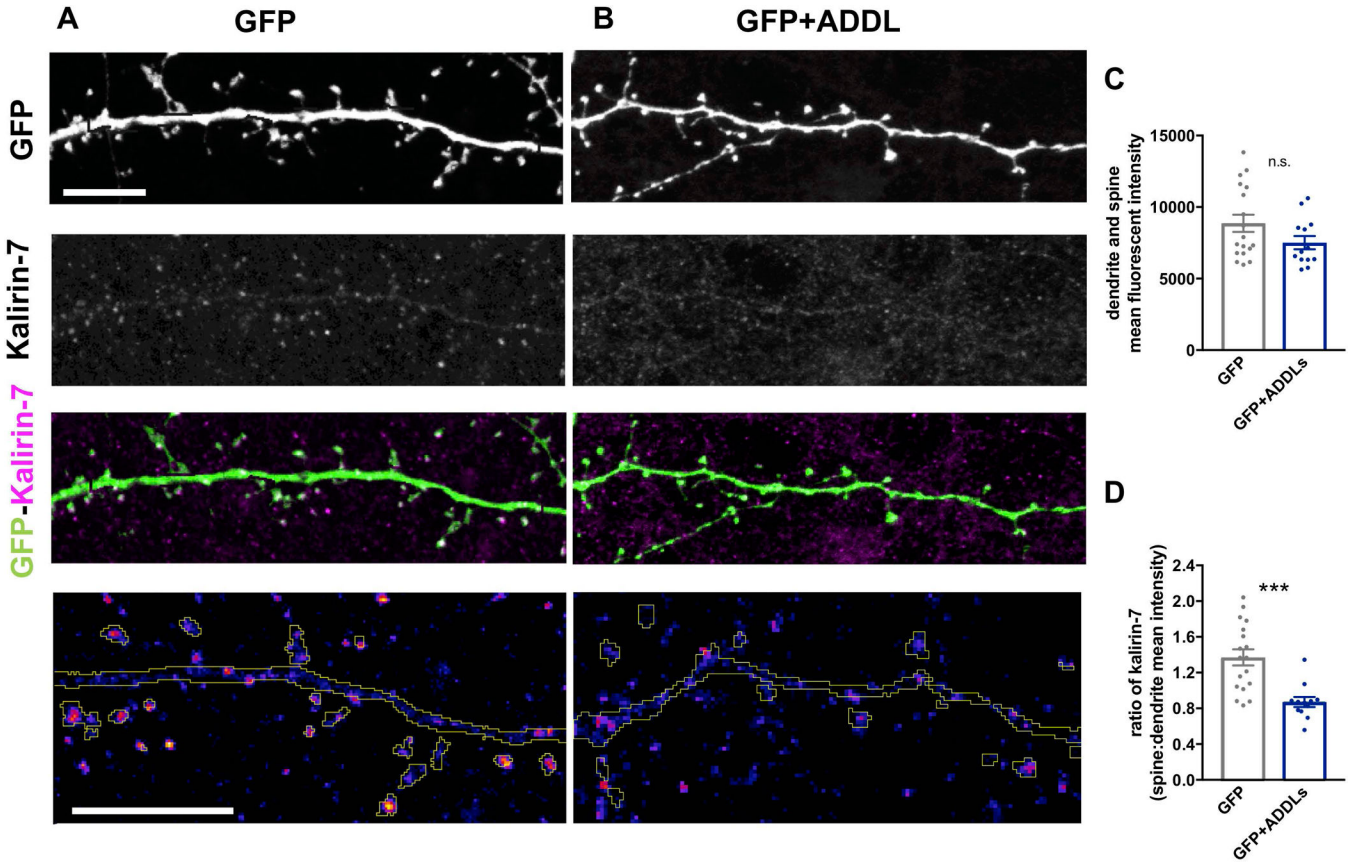

Fig. 2. Endogenous kalirin-7 in dendritic spine heads is rapidly reduced by ADDL treatment.

Rat cortical neurons (DIV 28) were treated with vehicle or 500 nM ADDLs for 2 h, then immunostained with antibodies against kalirin-7 and GFP. (a) Endogenous kalirin-7 is present throughout the dendrite and in the majority of dendritic spines. (b) Addition of ADDLs reduces endogenous kalirin-7 in spines. (c) The total levels of endogenous kalirin-7 following ADDL treatment is quantified by measuring mean intensity of kalirin-7 signal in dendrites and dendritic spines. (d) Ratio of endogenous kalirin-7 in spines:dendrites is reduced in ADDL-treated cultures. Data were collected from 13–18 dendrite segments from 5–7 neurons. Scale bar = 10 μm. ***p<0.001.

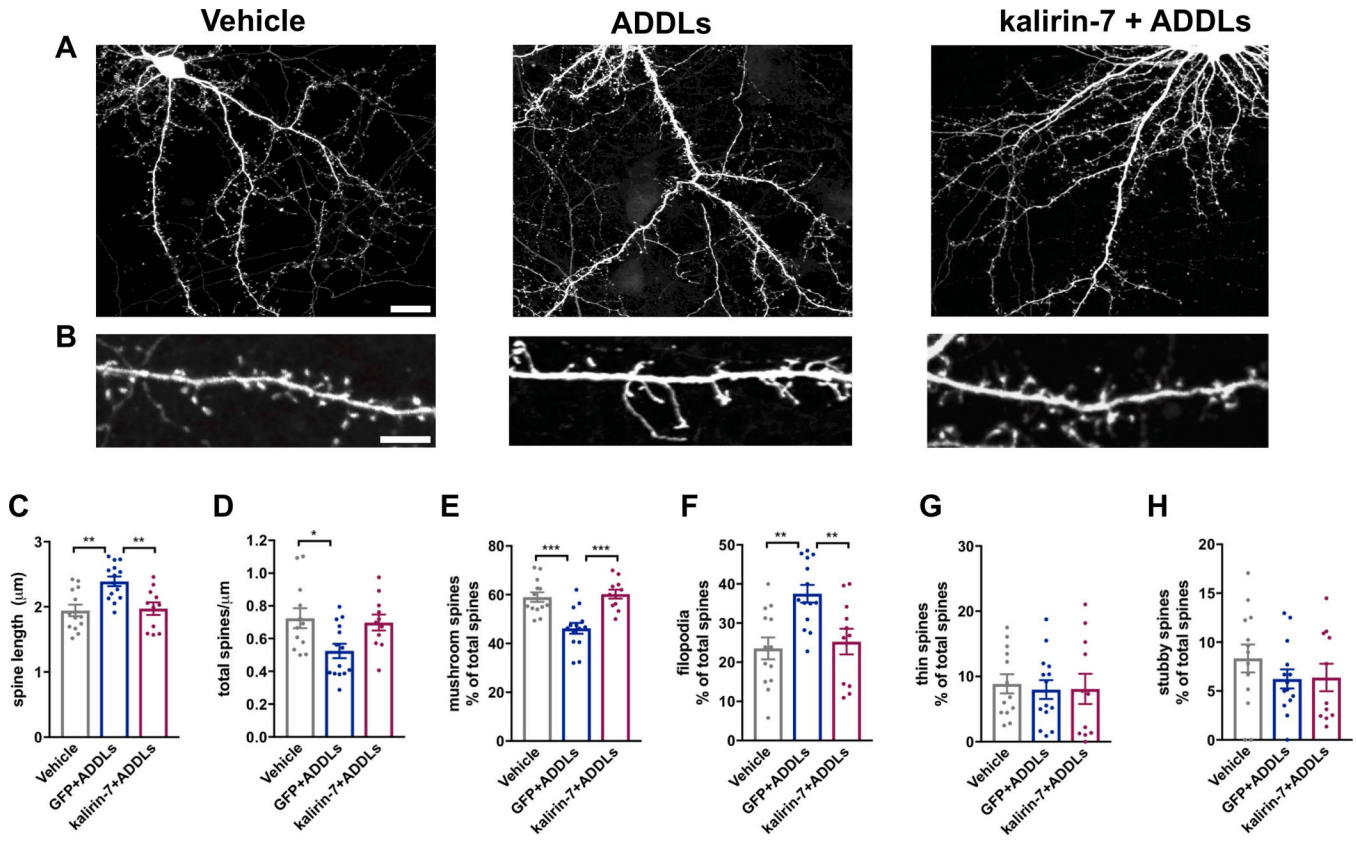

Fig. 3. Kalirin-7 protects against ADDL-inducted dendritic spine morphology in cortical neurons.

(a) Rat cortical neurons (DIV 28) were transfected with eGFP-N2 plasmid ± kalirin-7-myc plasmid and treated with vehicle or 500 nM ADDLs for 16 h. Scale bar = 20 μm. (b) High-magnification images of vehicle- and ADDL-treated neurons. Scale bar = 5 μm. (c) Addition of ADDLs increases dendritic spine length, an effect that is blocked by overexpression of kalirin-7. (d) ADDLs also decreased dendritic spine density; loss of dendritic spines was partially prevented by kalirin-7 overexpression. (e) Further analysis revealed that ADDL treatment reduced the percent of mushroom spines, but this effect is occluded by the overexpression of kalirin-7. (f) Filopodia represented a larger percentage of total spines in ADDL-treated neurons and this increase was occluded by kalirin-7 overexpression. (g-h) ADDLs did not alter thin or stubby spines. Means + SEMs, *p=0.02, **p<0.01, ***p<0.001. Data were collected from 2–6 dendrite segments totaling approximately 100 μm from 11–14 neurons.

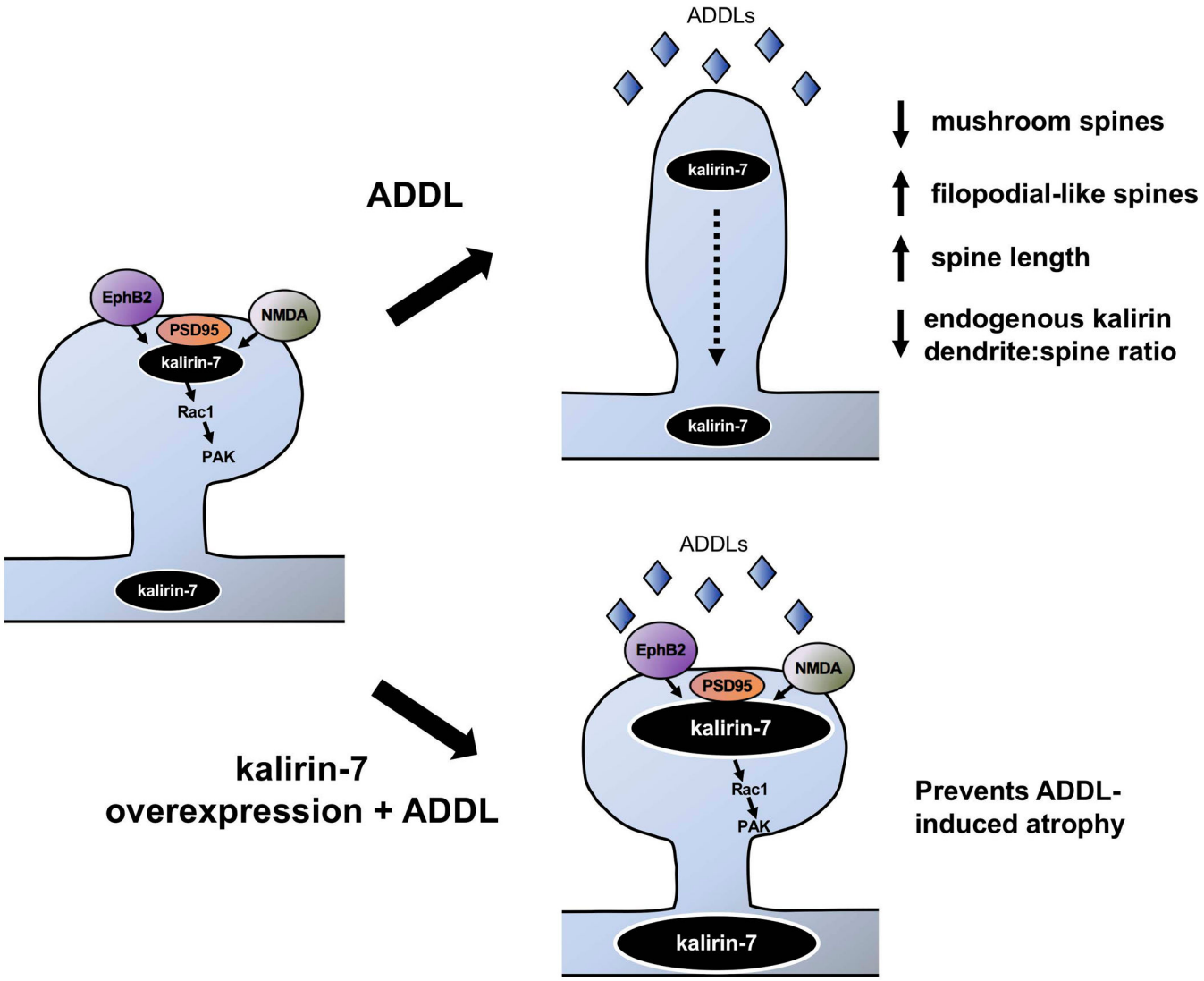

Fig. 4. Proposed model of kalirin-7 protection.

Under basal conditions kalirin-7 is localized to both the spines and the dendrites. Previous studies show that kalirin-7 colocalizes with PSD-95 and is activated by multiple mechanisms, including by EphB2 and NMDA receptors. Kalirin-7 can stabilize dendritic spines through Rac1-PAK-mediated signaling. Further, ADDLs reduce levels of PSD-95 as well as EphB2 and NMDA receptors. In the current manuscript, we show that ADDL treatment alters the equilibrium of endogenous kalirin-7 in spines compared to dendrites. ADDLs also induce an immature dendritic spine phenotype characterized by a loss of mushroom shaped spines and an increase filopodial-like spines and spine length. Overexpression of kalirin-7 prevents this ADDL-induced spine atrophy, suggesting that ADDLs destabilize spines by interfering with kalirin-7 localization and signaling.