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ORIGINAL PAPER

Role of Cdk5 in Kalirin7-Mediated Formation of Dendritic Spines

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Abstract
A majority of excitatory synapses in the brain are localized on the dendritic spines. Alterations of spine density and morphology are associated with many neurological diseases. Understanding the molecular mechanisms underlying spine formation is important for understanding these diseases. Kalirin7 (Kal-7) is localized to the postsynaptic side of excitatory synapses in the neurons. Overexpression of Kal-7 causes an increase in spine density whereas knockdown expression of endogenous Kal-7 results in a decrease in spine density in primary cultured cortical neurons. However, the mechanisms underlying Kal-7-mediated spine formation are not entirely clear. Cyclin-dependent kinase 5 (Cdk5) plays a vital role in the formation of spines and synaptic plasticity. Kal-7 is phosphorylated by CDK5 at Thr^{1590}, the unique Cdk5 phosphorylation site in the Kal-7 protein. This study was to explore the role of CDK5-mediated phosphorylation of Kal-7 in spine formation and the underlying mechanisms. Our results showed expression of Kal-7T/D (mimicked phosphorylation), Kal-7T/A mutants (blocked phosphorylation) or wild-type (Wt) Kal-7 caused in a similar increase in spine density, while spine size of Wt Kal-7-expressing cortical neurons was bigger than that in Kal-7 T\A-expressing neurons, but smaller than that in Kal-7T/D-expressing neurons. The fluorescence intensity of NMDA receptor subunit NR2B (GluN2B) staining was stronger along the MAP2 positive dendrites of Kal-7T/D-expressing neurons than that in Kal-7T/A- or Wt Kal-7-expressing neurons. The fluorescence intensity of AMPA receptor subunit GluR1 (GluA1) staining showed the same trend as GluN2B staining. These findings suggest that Cdk5 affects the function of Kal-7 on spine morphology and function via GluN2B and GluA1 receptors during dendritic spine formation.

Keywords Synapse · Spine formation · Kalirin · Phosphorylation · Primary cortical neuron

Introduction

Dendritic spines on which most excitatory synapses are localized are very dynamic and play an important role in synaptic plasticity, a process underlying learning and memory and other cognitive processes [1, 2]. Synaptic connections between neurons are essential for neuronal function in the central nervous system (CNS). Change of synaptic plasticity is often connected with alterations in spine density, size and morphology [3, 4]. AMPA and NMDA receptors that are colocalized at the postsynaptic membrane of most excitatory synapses are involved in dendritic spine formation, maintenance and remodeling [5]. For example, the synaptic NMDA receptor subunit GluN2B that is critically involved in the formation or maintenance of dendritic spines plays an essential role in long-term potentiation (LTP) induction, and overexpression of GluN2B enhanced LTP [6, 7]. AMPA receptors play a key role in regulating spine morphology [8]. Distribution of functional AMPA receptors is tightly correlated with spine geometry [9], and synaptic insertion of GluA1 is required to permit a stable increase in spine size [10]. The aberrant synaptic structure and connections are found in various psychiatric disorders and neurological diseases, such as autism and depression [11–13]. Alterations in dendritic spines in specific brain regions are associated with alterations of specific animal behaviors [14]. Alterations of spine density and morphology are linked to many neurological diseases and psychiatric disorders [11, 15–17].

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Therefore, better understanding of molecular mechanisms underlying spine formation and synaptogenesis will enhance our understanding of the mechanisms underlying various psychiatric disorders and neurological diseases.

Kalirin7 (Kal-7) is a major isoform of Kalirin in the adult rodent brain and plays an essential role in the formation of dendritic spines and excitatory synapses [15, 18, 19]. Dysregulation of Kal-7 expression is associated with many psychiatric diseases [15]. Kal-7, exclusively localized to the postsynaptic side of excitatory synapses in the brain, interacts with many postsynaptic density zone (PDZ) domain-containing proteins through its PDZ-binding motif [20, 21]. The expression of Kal-7 is markedly increased at the postnatal day 14, a time point of the starting maximal synaptogenesis in the rodent brain, and overexpression of Kal-7 increases spine density whereas knockdown of endogenous Kal-7 decreases spine density and excitatory synapse number [18, 21, 22]. Furthermore, Kal-7 deficient mice show a reduction in spine density and synapse number in the apical dendrites of hippocampal CA1 pyramidal neurons accompanied by a decrease in spontaneous excitatory postsynaptic current (EPSC) frequency and markedly blunted LTP [20]. However, the mechanisms through which Kal-7 regulates the spine formation, synaptic plasticity and synaptogenesis are largely unknown.

Cyclin-dependent kinases 5 (CdK5), a proline-directed serine/threonine kinases, plays a critical role in spine formation, synaptogenesis and synaptic plasticity [23–27]. Dysregulation of Cdk5 is associated with many neurological diseases [28]. The single Cdk5 phosphorylation site (Thr^{1590}) is localized between the GEF domain and PDZ binding motif on Kal-7 (Fig. 1a). Our previous study shows that the T/D (Asp) mutant mimics the phosphorylation of Cdk5, expression of Kal-7T/A (Ala) is unable to cause extension of protrusions in PC12 cells, Kal-7-mediated extension of broad cytoplasmic protrusions in PC 12 cells is largely eliminated by co-expression with domain negative (DN) Cdk5, but DN-Cdk5 fails to block the ability of Kal-7T/D to cause extension of protrusions [29]. Furthermore, the level of Cdk5 protein is decreased in the cortex of Kal-7 deficient mice [20]. This study was designed to investigate the role of Cdk5 in Kal-7-mediated spine formation and explore the mechanisms through which Cdk5-mediated phosphorylation of Kal-7 affects the spine formation.

Materials and Methods

Antibodies

The following primary antibodies were used: GluN2B antibody (06-600, 1:200, Rabbit, EMD Millipore), GluA1 (1:500, Rabbit, Upstate), MAP2 antibody (AB15452, 1:500, Chicken, EMD Millipore), Myc antibody (9E10, 1:20, mouse); GFP antibody (1:1000, Rat, Nacalai Tesque, Japan) and Kal-7 2958 [20].

Plasmids

The pEAK vector encoding His6-myc-tagged Kal-7 was constructed as described [30, 31]. The pEAK vector encoding myc-Kal-7T/D and T/A were constructed by changing Thr^{1590} to Ala^{1590} and Asp^{1590}, respectively (Quickchange; Stratagene, La Jolla, CA) in pEAK His6-myc-Kal-7 [29]. Kal-7T/D was used to mimic constitutively phosphorylated Kal-7 and the phosphorylation in Kal-7T/A was blocked.

Cultures of Cortical Neurons and Transfection

Animals

Timed pregnant adult female Sprague Dawley rats from Charles River Laboratories or Xian Jiaotong University were housed one per cage with a 14–10 h light–dark cycle with food and water available ad libitum. All experiments were conducted in accordance with the guidelines established by the Animal Care and Use Committee of the UCHC and Shaanxi Normal University.

Primary cortical neurons were prepared from embryonic day 18 Sprague Dawley rats as described in our previous study [21]. For studying dendritic spine in Fig. 2, freshly dissociated cells were transfected (Amaxa, Germany) with a vector encoding GFP or doubly transfected with a vector encoding GFP plus a vector encoding myc-Kal-7, myc-Kal-7T/D or myc-Kal-7T/A mutant as described [21]. The ratio of vectors encoding GFP to Kal-7 mutants or Kal-7 was 1:3, which ensured that most GFP-expressing neurons expressed Kal-7 or its mutants. For studying GluN2B and GluA1 in Fig. 3, freshly dissociated cells were transfected with a vector encoding myc-Kal-7, myc-Kal-7T/D or myc-Kal-7T/A mutant as described [21]. After transfection, neurons were plated and cultured as described [21]. All reagents were from Life Technologies.

Immunocytochemistry

Immunostaining of neurons was performed in cortical neurons at Div20 as described [21]. Briefly, neurons were fixed with 4% paraformaldehyde at room temperature for 18 min or cold methanol (− 20 °C) for 12 min. After 5 min in a blocking buffer containing 1%BSA, 5% normal donkey serum, 0.20% Triton X-100 followed by 55 min in a blocking buffer without Triton X-100 at room temperature, cells were doubly stained with antibodies specific to Myc and GFP or triply stained with antibodies specific to GluA1 or GluN2B and Myc and MAP2 overnight at

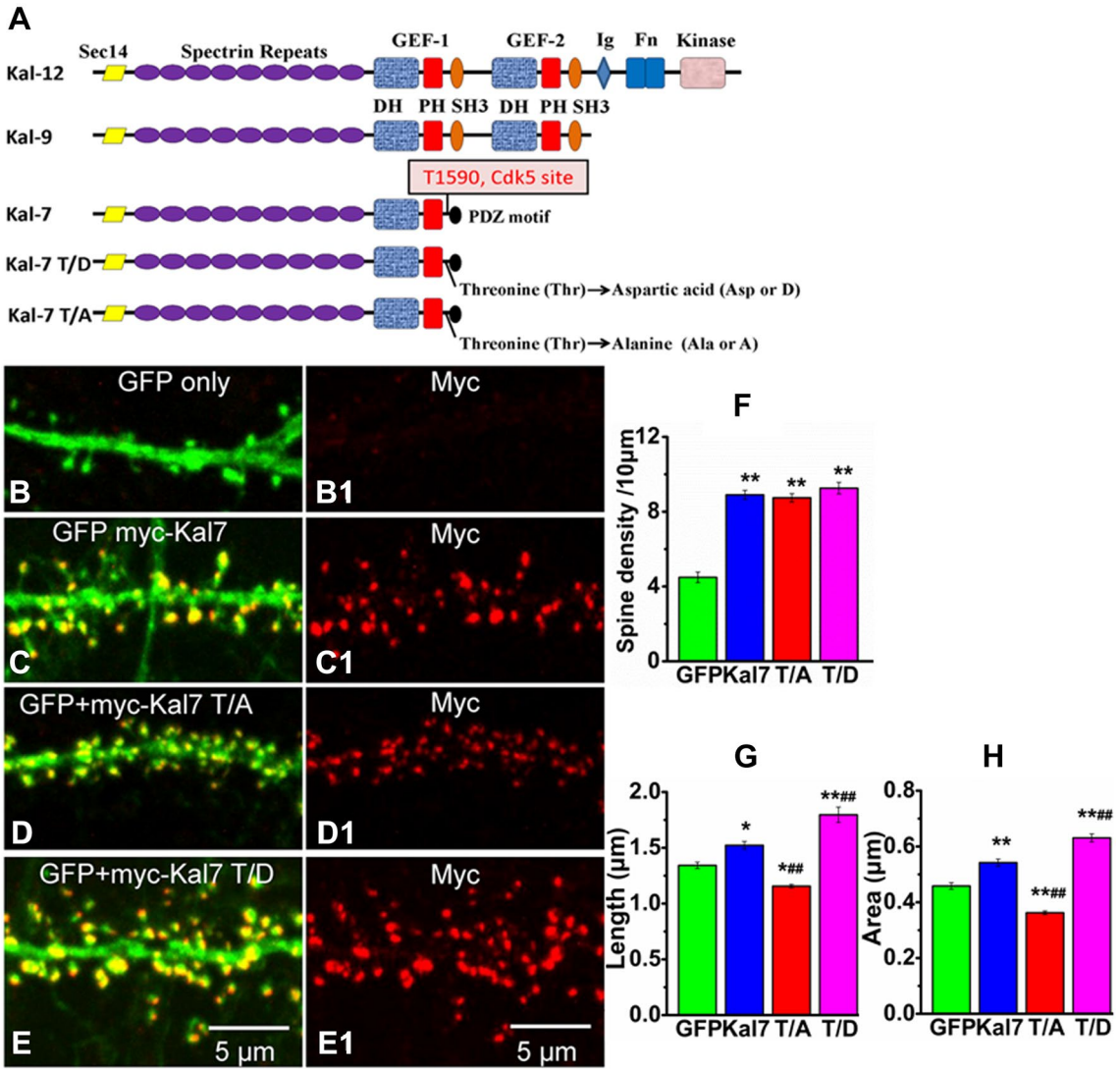

Fig. 1 The effect Thr1590 phosphorylation on dendritic spinesin primary cortical neurons. **a** Kalirin isoforms and Kal-7 mutant (T/D and T/A) constructs. The *Kalirin* gene produces multiple isoforms through alternative splicing and the use of multiple promoters. Kal-7 contains only one CDK5 site, T1590. T(Thr)/D (Asp) is a constitutively active mutant, and T(Thr)/A (Ala) is a dead mutant. Rat cortical neurons were transfected with a vector encoding GFP only (**b**), co-transfected with a vector encoding GFP and a vector encoding myc-Kal-7 (**c**), myc-Kal-7T/A (**d**) or myc-Kal-7T/D (**e**), respectively, at the time of plating. Neurons were fixed with 4% paraformaldehyde for double immunostaining with antibodies specific to Myc (red) and GFP (green) at DIV16. Myc staining was used to visualize expression of Kal-7 and its mutants (**b1**–**c1**), and GFP staining was used to intensify GFP signal. Myc positive clusters showed that Kal-7 and its mutants are most localized on the tip of dendritic spines. **f**–**h** are quantification of spine density (F, $F_{(3,57)}=80.50$, $^{**}p<0.01$ vs. GFP), spine length (**g** $F_{(3,1381)}=42.12$, $^{*}p<0.05$, $^{**}p<0.01$ vs. GFP, $^{\#\#}p<0.01$ vs. Kal-7) and spine area (**h** $F_{(3,1381)}=100.40$, $^{**}p<0.01$ vs. GFP, $^{\#\#}p<0.01$ vs. Kal-7), respectively. The data were analyzed by one way ANOVA followed by Tukey's post-hoc test. (Color figure online)

4 °C. Primary antibodies were visualized with appropriate secondary antibodies: Cy3-conjugated donkey anti-mouse IgG (Jackson Lab), Alexa Fluor 488-conjugated donkey anti-mouse IgG (Life Technology), Alexa Fluor 488-conjugated donkey anti-rabbit IgG (Life technology), Alexa Fluor 633-conjugated donkey anti-chicken IgG (Life technology). Images were visualized with a Zeiss LSM510 confocal microscope.

Image Analysis and Quantification

After the scale bar on the images was calibrated, spine density and the fluorescence intensity of GluA1 or GluN2B synaptic clusters were analyzed, and were limited to dendrites within 100 μm of the cell body using MetaMorph (Molecular Devices, Downington, PA) as described in our previous study [32]. Quantifications were performed by one

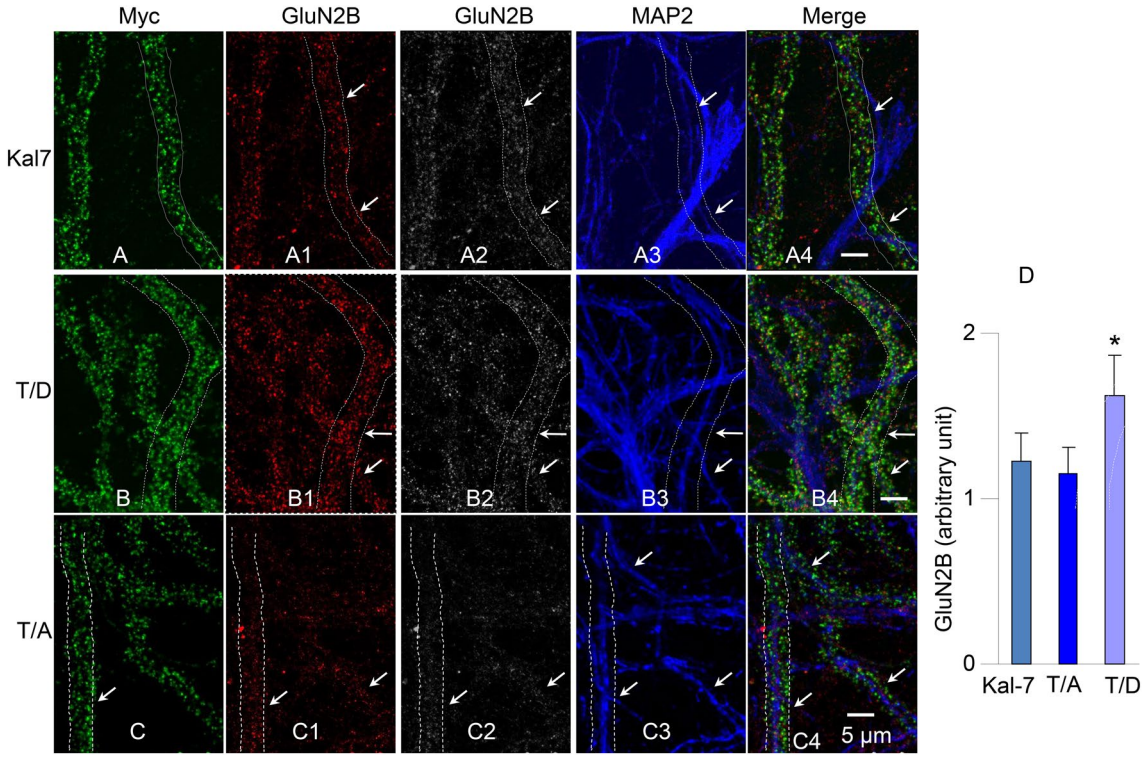

Fig. 2 Expression of Kal-7T/D, but not T/A mutant increased expression of GluN2B. Rat cortical neurons were transfected with a vector encoding myc-Kal-7 (**a**), myc-Kal-7T/D (**b**) or myc-Kal-7T/A (**c**) at the time of plating. Neurons were fixed for triple immunostaining with antibodies specific to Myc (green), GluN2B (**a1**–**c1** red; **a2**–**c**2 black and white) and MAP2 (**a3**–**c3** a dendritic marker, blue) at DIV 20. The data were analyzed by one way ANOVA followed by Tukey's post-hoc test. *$p < 0.05$ versus Kal-7 (**d**). (Color figure online)

researcher blind to the experiment. Data are shown as average ± SEM. Statistical analyses were performed using one way ANOVA followed by Tukey's post-hoc test.

Results

Expression of Kal-7T/D (Mimicking Phosphorylation) Mutant Increased Spine Size and Length While Expression of T/A Mutant Decreased Spine Size and Length

To study the effect of over-expression of Kal-7 and its CDK5 site mutants on spine density and spine morphology, freshly dissociated cortical neurons were doubly transfected with a vector encoding GFP plus a vector encoding Myc-Kal-7, Myc-Kal-7T/D mutant or Myc-Kal-7T/A mutant (1:3 ratio), respectively, on the day of neuronal preparation. As expected, analysis of co-expressing GFP and Kal-7 or its mutants showed that 90% GFP-expressing neurons were positive for Myc staining. Most dendritic spines have a clear spine head (Fig. 1b). GFP-expressing neurons transfected with the vector encoding GFP only were not stained for Myc (Fig. 1b1). As expected, over-expression of wild-type (Wt) Kal-7 resulted in a significant increase in spine density (Fig. 1c, f), spine length (Fig. 1c, g) and spine area (Fig. 1c, h) in comparison with the neurons expressing GFP only. Interestingly, expression of Kal-7T/A mutant decreased spine length (Fig. 1d, g) and spine area (Fig. 1d, h) compared with neurons expressing Wt Kal-7 (Fig. 1d, f–h). However, expression of Kal-7T/D mutant produced a significant increase in spine length (Fig. 1e, g) and spine area (Fig. 1e, h) in comparison with Wt Kal-7-expressing neurons.

Expression of Kal-7T/D, but not T/A Mutant Increased GluN2B Levels

To determine whether expression of Kal-7T/D and T/A mutant alters GluN2B expression, freshly dissociated cortical neurons were transfected with a vector encoding myc-Kal-7, myc-Kal-7T/D mutant or myc-Kal-7T/A mutant (1:3 ratio), respectively. Cortical neurons were triply stained with antibodies specific to myc (represents expression of exogenous Kal-7), MAP2 (a marker for dendrites) and NMDA receptor subunits GluN1 or GluN2B on Div20. Our findings indicated that the fluorescence intensity of GluN2B staining was stronger in Myc positive

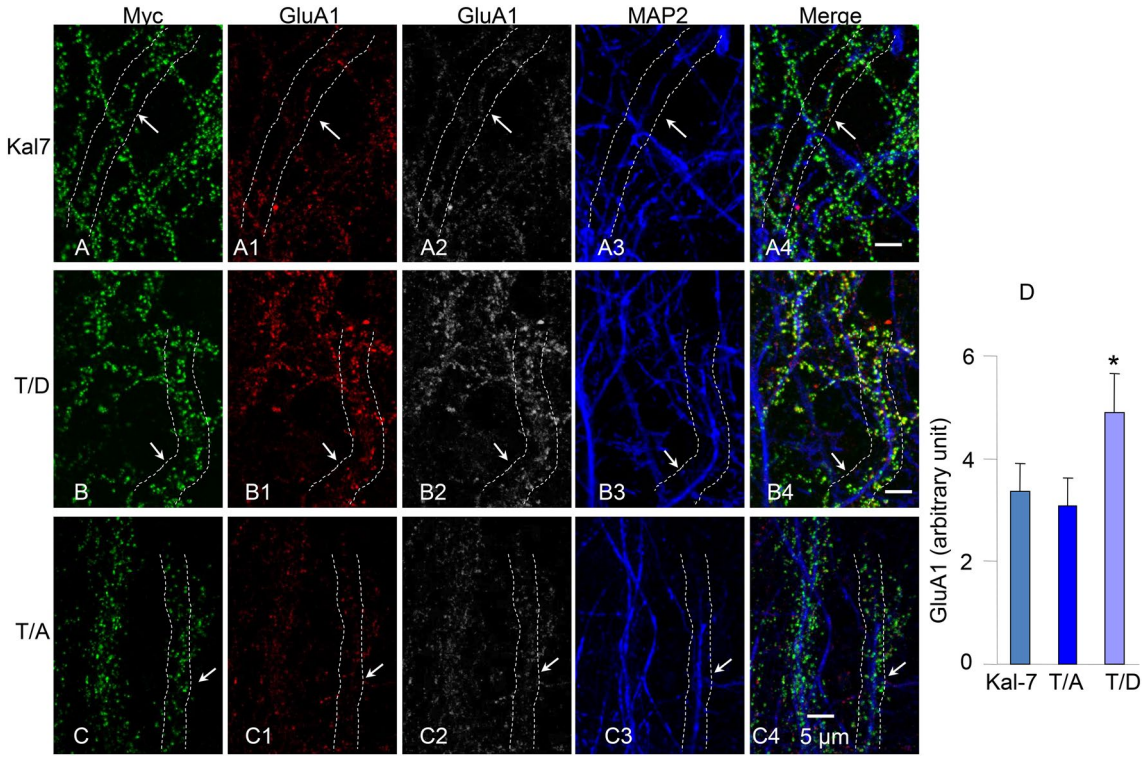

Fig. 3 Expression of Kal-7T/D, but not T/A mutant increased expression of GluA1. Rat cortical neurons were transfected with a vector encoding myc-Kal-7 (**a**), myc-Kal-7T/D (**b**) or myc-Kal-7T/A (**c**) at the time of plating. Neurons were fixed for triple immunostaining with antibodies specific to Myc (green), GluA1 (**a1**–**c1** red, **a2**–**c2** black and white) and MAP2 (**a3**–**c3** a dendritic marker, blue) at DIV 20. The data were analyzed by one way ANOVA followed by Tukey's post-hoc test. *$p < 0.05$ vs. Kal-7 (**d**). (Color figure online)

clusters along the dendrites of neurons expressing Kal-7T/D mutant than that along the dendrites of neurons expressing Kal-7T/A mutant (Fig. 2a–d); the fluorescence intensity of GluN1 staining was not altered (not shown).

Expression of Kal-7T/D, but not T/A Mutant Increased the Expression of GluA1 in Clusters Likely Present in Spines

To determine whether expression of Kal-7T/D and T/A mutants altered GluA1 expression, freshly dissociated cortical neurons were transfected with a vector encoding Kal-7, Kal-7T/D mutant or Kal-7T/A mutant (1:3 ratio), respectively. On Div20, cortical neurons were triply stained with antibodies specific to Myc, MAP2 and AMPA receptor subunit GluA1. Our data showed the fluorescence intensity of the GluA1 staining in Myc positive clusters along the dendrites of neurons expressing Kal-7T/D mutant significantly increased in comparison with neurons expressing Kal-7T/A or Wt Kal-7 (Fig. 3a–d).

Discussion

Phosphorylation of Kal-7 on Thr^{1590} Site by CDK5 Plays a Critical Role in Kal-7-Mediated Spine Morphology

Our previous study shows an Asp1590 mutation in Thr^{1590} (T/D) mimicked constitutively phosphorylated Kal-7 and an Ala1590 in Thr^{1590} (T/A) mutation blocked phosphorylation of Kal-7 [29]. The expression of Kal-7T/D mutant, T/A mutant or Wt Kal-7 produced a similar increase in spine density in cultured cortical neurons. However, the size and length of dendritic spine formed in cultured cortical neurons after Kal-7T/A expression are different from the size and length of dendritic spines formed after Wt Kal-7 or Kal-7T/D expression. Expression of Kal7 T/A caused a decrease in spine length and size compared with expression of Wt Kal7, whereas expression of Kal7 T/D increased spine size and length in cortical neurons compared with expression of Wt Kal7. These results showed

that mutation of the Cdk5 phosphorylation site in Kal-7 altered Kal-7 to influence spine size and length, but not density. These alterations in spine length and size may largely result from changes in the length of spine neck and the size of spine head. What is the significance of the altered size and length of dendritic spines? Synaptic function is associated with spine morphology and size [33, 34]. Increased size of dendritic spine [35] that contributes to LTP is associated with learning and memory [36, 37]. The size of a spine head is well correlated with the PSD size of a synapse [38]. Alterations in spine morphology are associated with synaptic plasticity, because the spine neck is an important structure for a headed spine to perform its normal function since the spine neck controls Ca^{2+} exchange between the head of dendritic spine and the shaft of the dendrite. This may prevent excitotoxicity to the dendrite and neuron by controlling excessive influxes of Ca^{2+} into the synapses in spine head [39, 40]. Larger spines harboring larger synapses contain more AMPA and NMDA receptors in the PSD and have stronger synaptic transmission [38, 41]. The size and geometry of the spine head and neck are important for a spine to coordinate and compartmentalize synaptic signaling, disruption of which could cause a variety of neurological diseases [42]. NMDA receptor-mediated calcium signaling compartmentalization relies strongly on the diameter and length of the spine neck [43]. These studies together with our results indicate that the phosphorylation of Thr^{1590} by CDK5 is important for Kal-7 to maintain the size of spine head and length of the spine neck, and support normal synaptic transmission and function. Similarly, previous studies show that CaMKII phosphorylation of Kal-7 at Thr95 is necessary for Kal-7 to increase spine size, but not spine density [44]. Expression of Kal-7 and Kal-7 T95A mutant caused a similar increase in spine density in cortical neurons compared to control. Meanwhile, expression of Kal-7 increased spine size but expression of Kal-7 T95A does not affect spine size. CaMKII phosphorylation of Kal-7 at Thr95 is sufficient to enhance expression of synaptic AMPA receptor subunit GluA1 and is required for LTP induction [44, 45]. These studies together with our results suggest phosphorylation of Kal-7 by Cdk5 or CaMKII play an important role in Kal-7-mediated alterations in spine formation and synaptic plasticity.

How the alterations of spine morphology are related to disease conditions? Changes in spine size, spine density and spine morphology are observed after stress, spatial training, in aging, an enriched environment, some psychiatric disorders and neurological diseases [12, 17, 46–49]. Cortical neurons in fragile-X syndrome and down syndrome show alterations in spine morphology characterized by a decrease in the stubby and mushroom spines and an increase in long, thin spines [50, 51]. Chronic cocaine treatments increase spine size, length and density accompanied by an increase in Kal-7 expression in the medium spiny neurons (MSNs) of the nucleus accumbens, a key drug reward area in the brain [52]. Decreased size of dendritic spines in the hippocampal CA3 neurons is accompanied by a reduction in Kal-7 levels in an animal model of depression compared with control animal [53]. Spine size in the MSNs of the striatum in schizophrenia is about 70% the spine size in healthy controls [54]. Decreased spine length in the deep layer III pyramidal neurons of the dorsolateral prefrontal cortex is related to a decrease in kalirin expression in subjects with schizophrenia [55, 56]. These studies together with our results raised a possibility that CDK5-mediated phosphorylation of Kal-7 is associated with alterations of spine morphology in various disease conditions. This question remains to be addressed in the future.

Thr^{1590} situated between the GEF domain and the PDZ binding motif [31] is shared by Kal-9, Kal-12 and the short Kal-5 isoform (Fig. 1a). Kal-9 and Kal-12 are mainly expressed in the brain during early development [30, 57] and are required for neurite outgrowth in young cortical neurons [57]. Over-expression of Kal-9 or Kal-12 in sympathetic neurons causes axon initiation and outgrowth [58]. Therefore, CDK5-mediated phosphorylation of Thr^{1590} may be critical for CDK5 to regulate the functions of Kal-5, Kal-9 and Kal-12, which may play a vital role in the axon outgrowth and dendrite development during early development, which is needed to be addressed in the future study.

The Role of GluN2B in Kal-7T/D- and T/A-Mediated Alterations in Spine Morphology

Our immunostaining results showed that Kal-7T/D positive clusters were overlapping with the distributions of GluN2B and GluA1, respectively in the postsynaptic side of excitatory synapses in both dendritic spines and shafts in cortical neurons, which is in line with our previous report [21]. Over-expression of Kal-7T/D lead to an increase in the florescent intensity of GluN2B positive clusters consistent with a synaptic localization compared with Wt Kal-7-expressing neurons. Interaction of GluN2B with Kal-7 in excitatory synapses is important for normal synaptic plasticity [59, 60]. GluN2B plays an important role in LTP induction, spine formation, synaptic plasticity, learning and memory [61, 62]. $Kal\text{-}7^{KO}$ mice show a decrease in both GluN2B-dependent NMDA receptor currents in cortical pyramidal neurons and GluN2B protein levels in the hippocampus [20]. These data suggest that GluN2B-containing NMDA receptors play a pivotal role in Kal7 T/D and T/A-mediated alterations in spine morphology during spine formation and synaptogenesis [60].

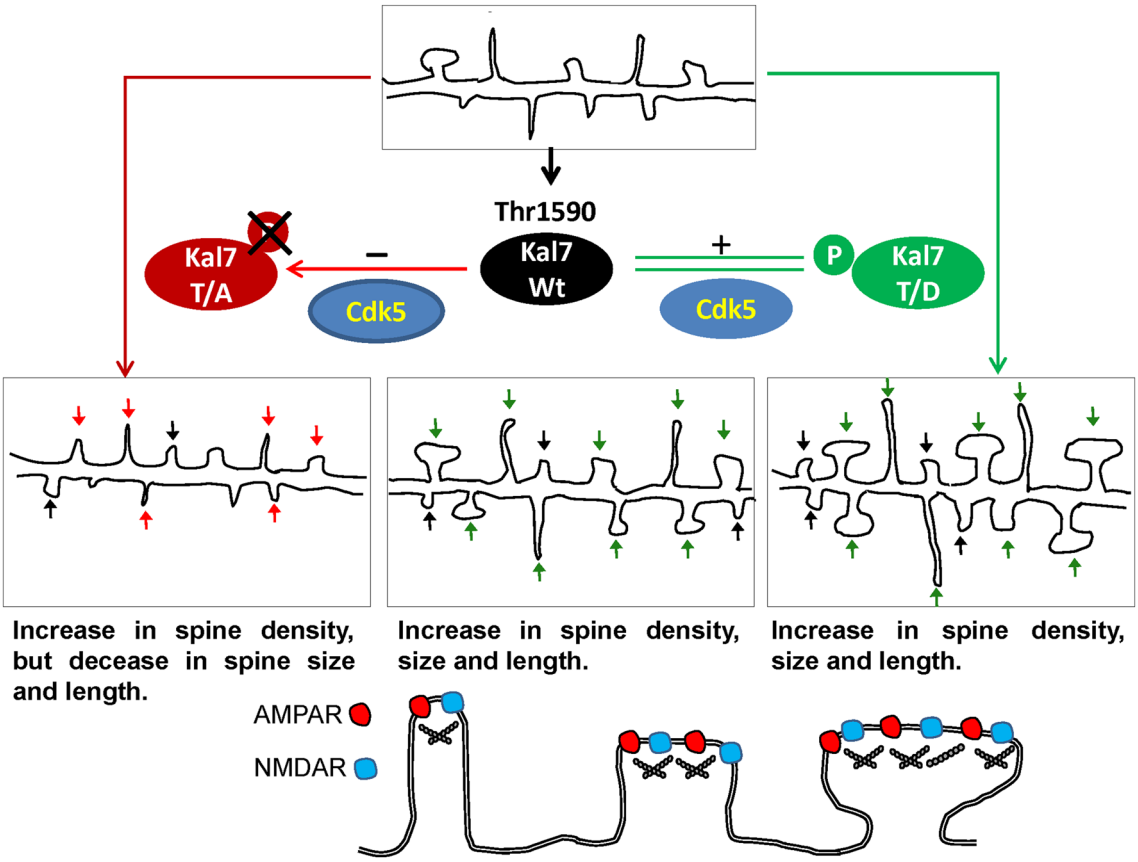

Fig. 4 Working model of Cdk5-mediated phosphorylation of Kal-7 in the formation of dendritic spines. Green, black and red arrowheads indicated expanded spines, new spines and shrunken spines, respectively. (Color figure online)

The Role of GluA1 in Kal-7T/D- and T/A-Mediated Alterations in Spine Morphology

Our results showed that Kal-7T/D positive clusters were overlapping with the distributions of GluA1 in the postsynaptic side of excitatory synapses along the dendrites of cortical neurons, in agreement with our previous report [21, 44]. Expression of Kal-7T/D mutant caused an overall increase in the florescent intensity of GluA1 clusters that likely correspond to spines head along the dendrites of cortical neurons in comparison with the neurons expressing Wt Kal-7. Synaptic insertion of GluA1 caused an increase in spine size [10]. Large dendritic spines are particularly enriched in GluA1 [63]. Kal7 that interacts with GluA1 is required for activity-dependent spine enlargement which may result from increased GluA1 content in spines [44]. LTP-induced increase in spine size is accompanied by recruiting GluA1 into spines [64–66]. These results indicate that the recruitment of GluA1 to spines/synapses may depend on Kal7 phosphorylation during spine formation.

These data confirm that Cdk5-mediated phosphorylation of Kal-7 is required for Kal-7 to affect spine morphology and synaptic function, via its effects on both GluN2B-containing NMDA receptors and GluA1-containing AMPA receptors (Fig. 4).

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References

1. Yuste R, Bonhoeffer T (2001) Morphological changes in dendritic spines associated with long-term synaptic plasticity. Annu Rev Neurosci 24:1071–1089. https://doi.org/10.1146/annurev.neuro.24.1.1071
2. Ehlers MD (2002) Molecular morphogens for dendritic spines. Trends Neurosci 25:64–67
3. Engert F, Bonhoeffer T (1999) Dendritic spine changes associated with hippocampal long-term synaptic plasticity. Nature 399:66–70
4. Yang Y, Zhou Q (2009) Spine modifications associated with long-term potentiation. Neuroscientist 15:464–476
5. McKinney RA (2010) Excitatory amino acid involvement in dendritic spine formation, maintenance and remodelling. J Physiol 588:107–116

6. Akashi K et al (2009) NMDA receptor GluN2B (GluR epsilon 2/NR2B) subunit is crucial for channel function, postsynaptic macromolecular organization, and actin cytoskeleton at hippocampal CA3 synapses. J Neurosci 29:10869–10882
7. Tang YP et al (1999) Genetic enhancement of learning and memory in mice. Nature 401:63–69. https://doi.org/10.1038/43432
8. Kasai H, Matsuzaki M, Noguchi J, Yasumatsu N, Nakahara H (2003) Structure-stability-function relationships of dendritic spines. Trends Neurosci 26:360–368
9. Matsuzaki M et al (2001) Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. Nat Neurosci 4:1086–1092
10. Kopec CD, Real E, Kessels HW, Malinow R (2007) GluR1 links structural and functional plasticity at excitatory synapses. J Neurosci 27:13706–13718
11. van Spronsen M, Hoogenraad CC (2010) Synapse pathology in psychiatric and neurologic disease. Curr Neurol Neurosci Rep 10:207–214. https://doi.org/10.1007/s11910-010-0104-8
12. Qiao H et al (2016) Dendritic spines in depression: what we learned from animal models. Neural Plast 2016:8056370. https://doi.org/10.1155/2016/8056370
13. Waites CL, Garner CC (2011) Presynaptic function in health and disease. Trends Neurosci 34:326–337. https://doi.org/10.1016/j.tins.2011.03.004
14. Gipson CD, Olive MF (2017) Structural and functional plasticity of dendritic spines—root or result of behavior? Genes Brain Behav 16:101–117. https://doi.org/10.1111/gbb.12324
15. Mandela P, Ma XM (2012) Kalirin, a key player in synapse formation, is implicated in human diseases. Neural Plast 2012:728161
16. Lai KO, Ip NY (2013) Structural plasticity of dendritic spines: the underlying mechanisms and its dysregulation in brain disorders. Biochim Biophys Acta 1832:2257–2263. https://doi.org/10.1016/j.bbadis.2013.08.012
17. Forrest MP, Parnell E, Penzes P (2018) Dendritic structural plasticity and neuropsychiatric disease. Nat Rev Neurosci 19:215–234. https://doi.org/10.1038/nrn.2018.16
18. Ma XM, Huang J, Wang Y, Eipper BA, Mains RE (2003) Kalirin, a multifunctional Rho guanine nucleotide exchange factor, is necessary for maintenance of hippocampal pyramidal neuron dendrites and dendritic spines. J Neurosci 23:10593–10603
19. Penzes P et al (2001) The neuronal Rho-GEF Kalirin-7 interacts with PDZ domain-containing proteins and regulates dendritic morphogenesis. Neuron 29:229–242
20. Ma XM et al (2008) Kalirin-7 is required for synaptic structure and function. J Neurosci 28:12368–12382. https://doi.org/10.1523/JNEUROSCI.4269-08.2008
21. Ma XM, Wang Y, Ferraro F, Mains RE, Eipper BA (2008) Kalirin-7 is an essential component of both shaft and spine excitatory synapses in hippocampal interneurons. J Neurosci 28:711–724. https://doi.org/10.1523/JNEUROSCI.5283-07.2008
22. Ichikawa M, Muramoto K, Kobayashi K, Kawahara M, Kuroda Y (1993) Formation and maturation of synapses in primary cultures of rat cerebral cortical cells: an electron microscopic study. Neurosci Res 16:95–103
23. Lai KO, Ip NY (2015) Cdk5: a key player at neuronal synapse with diverse functions. Mini Rev Med Chem 15:390–395
24. Hawasli AH et al (2007) Cyclin-dependent kinase 5 governs learning and synaptic plasticity via control of NMDAR degradation. Nat Neurosci 10:880–886. https://doi.org/10.1038/nn1914
25. Mita N, He X, Sasamoto K, Mishiba T, Ohshima T (2016) Cyclin-dependent kinase 5 regulates dendritic spine formation and maintenance of cortical neuron in the mouse brain. Cereb Cortex 26:967–976. https://doi.org/10.1093/cercor/bhu264
26. Mishiba T et al (2014) Cdk5/p35 functions as a crucial regulator of spatial learning and memory. Mol Brain 7:82. https://doi.org/10.1186/s13041-014-0082-x
27. Shah K, Rossie S (2018) Tale of the good and the Bad Cdk5: remodeling of the actin cytoskeleton in the brain. Mol Neurobiol 55:3426–3438. https://doi.org/10.1007/s12035-017-0525-3
28. Su SC, Tsai LH (2011) Cyclin-dependent kinases in brain development and disease. Annu Rev Cell Dev Biol 27:465–491. https://doi.org/10.1146/annurev-cellbio-092910-154023
29. Xin X et al (2008) Regulation of Kalirin by Cdk5. J Cell Sci 121:2601–2611
30. Johnson RC, Penzes P, Eipper BA, Mains RE (2000) Isoforms of kalirin, a neuronal Dbl family member, generated through use of different 5′- and 3′-ends along with an internal translational initiation site. J Biol Chem 275:19324–19333. https://doi.org/10.1074/jbc.M000676200
31. Penzes P et al (2000) An isoform of kalirin, a brain-specific GDP/GTP exchange factor, is enriched in the postsynaptic density fraction. J Biol Chem 275:6395–6403
32. Ma XM et al (2011) Kalirin-7, an important component of excitatory synapses, is regulated by estradiol in hippocampal neurons. Hippocampus 21:661–677. https://doi.org/10.1002/hipo.20780
33. Blanpied TA, Ehlers MD (2004) Microanatomy of dendritic spines: emerging principles of synaptic pathology in psychiatric and neurological disease. Biol Psychiatry 55:1121–1127. https://doi.org/10.1016/j.biopsych.2003.10.006
34. Yuste R, Bonhoeffer T (2001) Morphological changes in dendritic spines associated with long-term synaptic plasticity. Annu Rev Neurosci 24:1071–1089
35. Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H (2004) Structural basis of long-term potentiation in single dendritic spines. Nature 429:761–766. https://doi.org/10.1038/nature02617
36. De Roo M, Klauser P, Garcia PM, Poglia L, Muller D (2008) Spine dynamics and synapse remodeling during LTP and memory processes. Prog Brain Res 169:199–207. https://doi.org/10.1016/S0079-6123(07)00011-8
37. Segal M (2017) Dendritic spines: morphological building blocks of memory. Neurobiol Learn Mem 138:3–9. https://doi.org/10.1016/j.nlm.2016.06.007
38. Bourne JN, Harris KM (2008) Balancing structure and function at hippocampal dendritic spines. Annu Rev Neurosci 31:47–67. https://doi.org/10.1146/annurev.neuro.31.060407.125646
39. Nimchinsky EA, Sabatini BL, Svoboda K (2002) Structure and function of dendritic spines. Annu Rev Physiol 64:313–353. https://doi.org/10.1146/annurev.physiol.64.081501.160008
40. Segal M (1995) Dendritic spines for neuroprotection: a hypothesis. Trends Neurosci 18:468–471
41. El-Husseini AE, Schnell E, Chetkovich DM, Nicoll RA, Bredt D (2000) S. PSD-95 involvement in maturation of excitatory synapses. Science 290:1364–1368
42. Ebrahimi S, Okabe S (2014) Structural dynamics of dendritic spines: molecular composition, geometry and functional regulation. Biochim Biophys Acta 1838:2391–2398. https://doi.org/10.1016/j.bbamem.2014.06.002
43. Noguchi J, Matsuzaki M, Ellis-Davies GC, Kasai H (2005) Spine-neck geometry determines NMDA receptor-dependent Ca^{2+} signaling in dendrites. Neuron 46:609–622. https://doi.org/10.1016/j.neuron.2005.03.015
44. Xie Z et al (2007) Kalirin-7 controls activity-dependent structural and functional plasticity of dendritic spines. Neuron 56:640–656
45. Herring BE, Nicoll RA (2016) Kalirin and Trio proteins serve critical roles in excitatory synaptic transmission and LTP. Proc Natl Acad Sci USA 113:2264–2269. https://doi.org/10.1073/pnas.1600179113
46. Long LH et al (2009) Age-related synaptic changes in the CA1 stratum radiatum and spatial learning impairment in rats. Clin Exp Pharmacol Physiol 36:675–681. https://doi.org/10.1111/j.1440-1681.2008.05132.x

47. Moser MB, Trommald M, Andersen P (1994) An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. Proc Natl Acad Sci USA 91:12673–12675
48. Berman RF, Hannigan JH, Sperry MA, Zajac CS (1996) Prenatal alcohol exposure and the effects of environmental enrichment on hippocampal dendritic spine density. Alcohol 13:209–216
49. Fiala JC, Spacek J, Harris KM (2002) Dendritic spine pathology: cause or consequence of neurological disorders? Brain Res Brain Res Rev 39:29–54
50. Irwin SA, Galvez R, Greenough WT (2000) Dendritic spine structural anomalies in fragile-X mental retardation syndrome. Cereb Cortex 10:1038–1044
51. Kaufmann WE, Moser HW (2000) Dendritic anomalies in disorders associated with mental retardation. Cereb Cortex 10:981–991
52. Kiraly DD et al (2010) Behavioral and morphological responses to cocaine require kalirin7. Biol Psychiatry 68:249–255. https://doi.org/10.1016/j.biopsych.2010.03.024
53. Xu C et al (2016) Orbitofrontal cortex 5-HT2A receptor mediates chronic stress-induced depressive-like behaviors and alterations of spine density and Kalirin7. Neuropharmacology 109:7–17. https://doi.org/10.1016/j.neuropharm.2016.02.020
54. Roberts RC, Conley R, Kung L, Peretti FJ, Chute DJ (1996) Reduced striatal spine size in schizophrenia: a postmortem ultrastructural study. Neuroreport 7:1214–1218
55. Konopaske GT, Lange N, Coyle JT, Benes FM (2014) Prefrontal cortical dendritic spine pathology in schizophrenia and bipolar disorder. JAMA Psychiatry 71:1323–1331. https://doi.org/10.1001/jamapsychiatry.2014.1582
56. Hill JJ, Hashimoto T, Lewis DA (2006) Molecular mechanisms contributing to dendritic spine alterations in the prefrontal cortex of subjects with schizophrenia. Mol Psychiatry 11:557–566
57. Yan Y, Eipper BA, Mains RE (2015) Kalirin-9 and Kalirin-12 play essential roles in dendritic outgrowth and branching. Cereb Cortex 25:3487–3501. https://doi.org/10.1093/cercor/bhu182
58. May V, Schiller MR, Eipper BA, Mains RE (2002) Kalirin Dbl-homology guanine nucleotide exchange factor 1 domain initiates new axon outgrowths via RhoG-mediated mechanisms. J Neurosci 22:6980–6990
59. Kiraly DD, Lemtiri-Chlieh F, Levine ES, Mains RE, Eipper BA (2011) Kalirin binds the NR2B subunit of the NMDA receptor, altering its synaptic localization and function. J Neurosci 31:12554–12565. https://doi.org/10.1523/JNEUROSCI.3143-11.2011
60. Lemtiri-Chlieh F et al (2011) Kalirin-7 is necessary for normal NMDA receptor-dependent synaptic plasticity. BMC Neurosci 12:126. https://doi.org/10.1186/1471-2202-12-126
61. Brigman JL et al (2010) Loss of GluN2B-containing NMDA receptors in CA1 hippocampus and cortex impairs long-term depression, reduces dendritic spine density, and disrupts learning. J Neurosci 30:4590–4600. https://doi.org/10.1523/JNEUROSCI.0640-10.2010
62. Paoletti P, Bellone C, Zhou Q (2013) NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. Nat Rev Neurosci 14:383–400. https://doi.org/10.1038/nrn3504
63. Shinohara Y, Hirase H (2009) Size and receptor density of glutamatergic synapses: a viewpoint from left-right asymmetry of CA3-CA1 connections. Front Neuroanat 3:10. https://doi.org/10.3389/neuro.05.010.2009
64. Kessels HW, Malinow R (2009) Synaptic AMPA receptor plasticity and behavior. Neuron 61:340–350. https://doi.org/10.1016/j.neuron.2009.01.015
65. Mitsushima D, Ishihara K, Sano A, Kessels HW, Takahashi T (2011) Contextual learning requires synaptic AMPA receptor delivery in the hippocampus. Proc Natl Acad Sci USA 108:12503–12508. https://doi.org/10.1073/pnas.1104558108
66. Rumpel S, LeDoux J, Zador A, Malinow R (2005) Postsynaptic receptor trafficking underlying a form of associative learning. Science 308:83–88. https://doi.org/10.1126/science.1103944