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Reelin attenuates the development of inhibitory synapse in primary hippocampal neurons

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ABBREVIATIONS

DMSO	Dimethylsulfoxide
PBS	Phosphate-buffered saline
TBS	Tris-buffered saline
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
GABA	γ-aminobutyric acid
ApoER2	Apolipoprotein E receptor 2
VLDLR	Very low density lipoprotein receptor
MAPK	Mitogen-activated protein kinase
ERK1/2	Extracellular signal-related kinase1/2
NC	Nitrocellulose
RIPA	Radioimmunoprecipitation assay
PFA	Paraformaldehyde
NGS	Normal goat serum
VGAT	Vesicular GABA transporter
GAD	Glutamate decarboxylase
MAP 2	Microtubule-associated protein 2
PI3-kinase	Phosphatidylinositol 3-kinase
SOM	Somatostatin

국 문 초 록

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이 성 은

지 도 교 수: 이 금 화

약학과

조선대학교 대학원

억제성 신경세포는 억제성 시냅스(inhibitory synpase)를 통해 흥분성 신경세포의 활성을 조절함으로써 신경망(neuronal network)을 안정화하고 항상성을 유지한다. 따라서 억제성 신경세포의 기능 장애는 신경의 흥분과 억제의 비균형을 초래하여 정보를 처리하는 과정에 문제를 일으키고 자폐증과 조현병, 우울증 등의 신경학적 질환을 초래한다. Reelin 은 분비성 당단백질로서 뇌의 피질발생 과정 중 신경세포의 이동을 조절하고 출생 후에는 수상돌기와 흥분성 시냅스의 발달에 관여한다. 현재까지 Reelin 에 대한 대부분의 연구가 흥분성 신경세포에 초점이 맞추어져 있어 억제성 시냅스 발달에 미치는 Reelin 의 역할에 대해서는 알려진

바가 거의 없다. 본 연구에서는 먼저 억제성 신경세포 내의 Reelin 이 유도하는 신호전달 경로의 활성화를 조사하였다. PI3K 신호전달 경로는 Reelin 에 의해 활성화되는 경로로 잘 알려져 있으나 실험 결과 흥분성 신경세포와 달리 억제성 신경세포에서는 그 활성화가 나타나지 않았다. 그럼에도 불구하고, Reelin 이 초대 해마신경세포에서 억제성 시냅스 후부의 단백질인 Gephyrin 집합체의 강도와 면적을 감소시키는 것으로 나타났다. 이것은 Reelin 이 억제성 신경세포에서 PI3K 신호전달 경로를 거치지 않는 간접적인 방법이나 혹은 다른 신호전달을 통해 억제 시냅스 발달에 영향을 미친다는 것을 제시한다. 덧붙여, 억제성 시냅스의 조절에 대한 Reelin 의 분자 기전을 연구하기 위해 초대 대뇌피질 신경세포에서 억제성 시냅스 발달에 관여하는 대표적인 단백질 또는 mRNA 의 발현을 비교·분석하였다. 본 연구는 GABA 성 억제성 신경세포에서 Reelin 의 새로운 역할을 밝히고, Reelin-관련 신경학적 질환의 분자적 기전을 규명하는데 기여한다.

Ⅰ. INTRODUCTION

GABAergic neurons confer inhibitory neurotransmission on neural circuits of the brain using γ-aminobutyric acid (GABA) through inhibitory synapses. The inhibitory neurotransmission reduces synaptic activation and controls the frequency of action potential firing in the postsynaptic neurons, which is essential for the balance of excitation/inhibition in neural information processing. Due to the role of inhibitory synapses as a platform for the neurotransmission in GABAergic circuits, the morphological and compositional alteration of inhibitory synapses is associated with neurological and neuropsychiatric diseases such as autism spectrum-related disorders, schizophrenia, and epilepsy [4, 5, 7, 14, 17, 26].

Reelin is a large glycoprotein which is secreted from marginal zone during corticogenesis in the fetal brain and then, is released from a group of inhibitory neurons after birth [2, 8]. Reelin has been originally discovered as a regulator of neuronal migration during the corticogenesis [16]. Further studies have elucidated the role of Reelin in synaptic plasticity as well as dendrite development and spinogenesis of excitatory neurons during postnatal days [15, 21, 22, 28]. Together, Reelin is a strong candidate gene for several psychiatric disorders such as schizophrenia, major depression, and autism spectrum disorders [6, 9, 11, 16]. In accordance with the various roles of Reelin, the intracellular signaling pathways activated by Reelin have been examined. Reelin binds to apolipoprotein E receptor 2 (ApoER2) and very low density lipoprotein receptor (VLDLR) to activate C3G/Rap1 for migration of neurons or to activate PI3K/Akt/mTor for dendrite and spine development [16]. Recently, Reelin has been reported to be involved in synaptic

plasticity by participating in the activation of MAPK/Erk1/2 through unknown receptor [15, 16, 27, 28]. While the vast majority of study of Reelin has been focused on excitatory neurons, much less is known about the role of Reelin in inhibitory synapse development. Previous studies have reported the positioning and neurites branching are altered by the deficiency of Reelin, apparently because of the migration defect of excitatory neurons [24, 29]. However, since Reelin has been uncovered to influences the postnatal development of neurons using different intracellular machineries from the one during prenatal corticogenesis, it has been questioned about the role of Reelin in the function of inhibitory neurons during postnatal days.

In this study, we investigated the activation of intracellular signalings induced by Reelin in inhibitory neurons. For the study of inhibitory synapse development, we analyzed the intensity, area, and density of Gephyrin or VGAT clusters in primary hippocamapal neurons. Furthermore, we examined the expression of proteins or mRNAs which regulate inhibitory synapse development, to understand potential molecular mechanisms of Reelin on inhibitory synapse regulation in primary cortical neurons.

Ⅱ. MATERIALS AND METHODS

1. Reagents and Antibodies

The primary antibodies used in this study were rabbit anti-phospho-S6 (Cell Signaling, #4858), rabbit anti-phospho-Akt (Cell Signaling, #4060), rabbit anti-Akt (Cell signaling, #4691), chicken anti-Map2 (Abcam, ab5392), mouse anti-Gephyrin (Synaptic systems, 147-021), anti-Gephyrin (Synaptic systems, 147-111), rabbit anti-VGAT (Synaptic Systems, 131 002), mouse anti-GAD67 (Millipore, MAB5406), anti-GAD65 (Abcam, ab26113), and mouse anti- β-actin-HRP (Sigma, A3854). The secondary antibodies were HRP-conjugated anti-rabbit and anti-mouse (Invitrogen, G21040, G21234), antibodies conjugated to AlexaFluor 488 goat anti-chicken (Invitrogen, A11039), AlexaFluor 555 goat anti-mouse (Invitrogen, A21422), and AlexaFluor 647 goat anti-rabbit (Invitrogen, A21244).

2. Primary neuronal culture

The dissociated cerebral cortices and hippocampi from the brain of embryonic day (E) 15.5 ICR mice were digested with 0.25% Trypsin (Gibco)/DNase I (Sigma) at 37℃ for 20 minutes; trypsin was inactivated using fetal bovine serum (invitrogen). Cells were placed on poly-L-lysine (Sigma) pre-coated plates and cultured in Neurobasal medium supplemented with 2% B27 supplement, Glutamax, and penicillin/streptomycin (Invitrogen). The neurons were cultured at 37℃ in a saturated atmosphere containing 95% air and 5% CO_2, and the medium was replaced with fresh medium a day after isolation. Afterward half of the medium was replaced every 3-4 days.

3. Production of recombinant Reelin and semi-purification

HEK293T cells were cultured in Dulbecco modified Eagle medium (Hyclone) with 10% fetal bovine serum and transfected with mock or Reelin cDNA construct pCrl, kindly provided by G. Darcangelo [8], using Transit (Mirus). The medium of the transfected cells was replaced with serum-free medium at 24 hours after transfection and then was collected for 2 days. The supernatants were centrifuged for the removal of cell debris and concentrated using Amicon Ultra-15 filters (Merck) at 2,680x g for 20 minutes prior to addition to neuronal cultures as described previously [22].

4. RNA isolation and RT-PCR analysis

Total RNA was abtained from primary neurons using GeneJET RNA Purification Kit (Thermo Scientific) according to the manufacturer's instructions. The total RNA (0.5-1μg) was reverse-transcribed to generate cDNA using the iScript^{TM} cDNA Synthesis Kit (bio-rad). The mRNA quantification was performed by StepOne real-time PCR system (Applied Biosystems) using a SYBR Green PCR Master Mix (Applied Biosystems). Transcript-specific primers used in the quantitative RT-PCR were: Gad67, F: 5'-CACAGGTCACCCTCGATTTTT-3', R: 5'- ACCATCCAACGATCTCTCTCATC-3', PrimerBank ID: 31982847a1; Gad65, F: 5'-TCCGGCTTTTGGTCCTTC-3', R: 5'-ATGCCGCCCGTGAACTTTT-3', PrimerBank ID: 6679925a1; Neuroligin1, F: 5'-GGTACTTGGCTTCTTGAGCAC-3', R: 5'- AAACACAGTGATTCGCAAGGG-3', PrimerBank ID: 28972598a1; Neuroligin2, 5'-TGTCATGCTCAGCGCAGTAG-3', 5'-GGTTTCAAGCCTATGTGCAGAT-3', PrimerBank ID: 33989614a1 ; Semaphorin 4D, F: 5'-CCTGGTGGTAGTGTTGAGAAC-3', R: 5'-GCAAGGCCGAGTAGTTAAAGAT-

3’, PrimerBank ID: 7305471a1; FGF7, F: 5’-TGGGCACTATATCTCTAGCTTGC-3’, R: 5’-GGGTGCGACAGAACAGTCT-3’, PrimerBank ID: 227116301c1; glyceraldehyde 3-phosphate dehydrogenase (Gapdh), F: 5’- AGGTCGGTGTGAACGGATTTG-3’, R: 5’-TGTAGACCATGTAGTTGAGGTCA-3’, PrimerBank ID: 9055212a1F. GAPDH was used as a reference gene for normalization. The relative quantification of the mRNA was calculated using the Pfaff1 method [23].

5. Western Blot Analysis

Primary cortical neurons were lysed at designated times with radioimmunoprecipitation assay buffer (RIPA). The protein extracts were separated using 6 or 8% SDS-PAGE gels and transferred to a 0.22-μm nitrocellulose (NC) membrane. The membrane was incubated in 5% skim milk blocking buffer for 1 hour at room temperature and then with a primary antibody at 4℃ overnight. After washing with Tris-buffered saline and Tween 20 (TBST), a secondary antibody was supplemented to the membrane for 1 hour at room temperature. Membranes were developed with western blot ECL solution (Supex). β-actin was used as an immunoblotting control.

6. Immunofluorescence

Primary hippocampal neurons, grown on glass coverslips coated with poly-L-lysine, were fixed in 4% paraformaldehyde (PFA)/phosphate buffered saline (PBS) for 15 minutes and then permeabilized by 0.1% Triton X-100/PBS for 10 minutes. After incubation with 10% normal goat serum (NGS) blocking buffer for 1 hour at room temperature, the neurons

were incubated with primary antibody at 4℃ overnight. After washing with PBS, the neurons on the coverslip were incubated with secondary antibodies for 1 hour at room temperature. The neurons were washed with PBS 3 times for 5 minutes and then were mounted on positively charged glass slides with mounting media (Thermo Scientific, 9990402). The neurons were imaged using a Nikon A1 confocal laser microscope system.

7. Quantification of Gephyrin / VGAT-positive clusters

The density, area and intensity of Gephyrin or Gephyrin/VGAT-positive clusters were measured from images of secondary dendrites of excitatory hippocampal neurons using the ImageJ program and NeuronJ plugin. The background intensity of each channel was subtracted and the function Adjust/Threshold in ImageJ was applied to each image with the same condition for quantifying cluster size. Thirty to fifty dendritic fields were analyzed from randomly selected pyramidal neurons in each experiment. All analyses were performed by an experimenter blinded with respect to treatment.

8. Statistical analysis

The density, area, and intensity of Gephyrin or Gephyrin/VGAT-positive clusters were measured from images of secondary dendrites of excitatory hippocampal neurons using the ImageJ program and NeuronJ plugin. The background intensity of each channel was subtracted and the function Adjust/Threshold in ImageJ was applied to each image with the same condition for quantifying cluster size. Thirty to fifty dendritic fields were analyzed from randomly selected pyramidal neurons in each experiment. All analyses were performed by an experimenter blinded with respect to treatment.

III. RESULTS

1. Activation of PI3K/S6 pathway by Reelin was not occurred in inhibitory neurons.

It is well-known that Reelin binds to ApoER2/VLDLR receptors and subsequently activates PI3K/Akt/mTor/S6K1 signaling pathway to enhance dendrite and spine development in neurons [13, 22]. Western blotting analysis showed that Reelin activated PI3K pathway by phosphorylating Akt and a downstream effector, S6, 15-30 minutes after Reelin treatment in 5-6 days *in vitro* primary cortical neurons (Fig. 1A). The phosphorylated level of Akt and S6 were 1.72 (0.56) and 1.51 (0.37), respectively, compared to mock-treated control (Fig. 1B). The phosphorylated level of the signaling proteins, shown in western blotting, does not present the response of individual neurons to Reelin. Since inhibitory neurons account for approximately 20% of total neurons, distinct response of inhibitory neurons to stimuli could be masked by the response of excitatory neurons which comprise the majority of neurons. To investigate whether canonical pathway of Reelin is activated in inhibitory neurons, we performed immunofluorescence using GAD67, a marker of inhibitory neuron, and phospho-S6 antibodies in primary hippocampal neurons. GAD67-positive/Map2-positive neurons were regarded as inhibitory neurons and GAD67-negative/Map2-positive neurons were considered as excitatory neurons. Surprisingly, the intensity of phosphorylated S6 in inhibitory neurons was not elevated in response to Reelin, while that in excitatory neurons was increased by 40.03% (Fig. 1C and 1D).

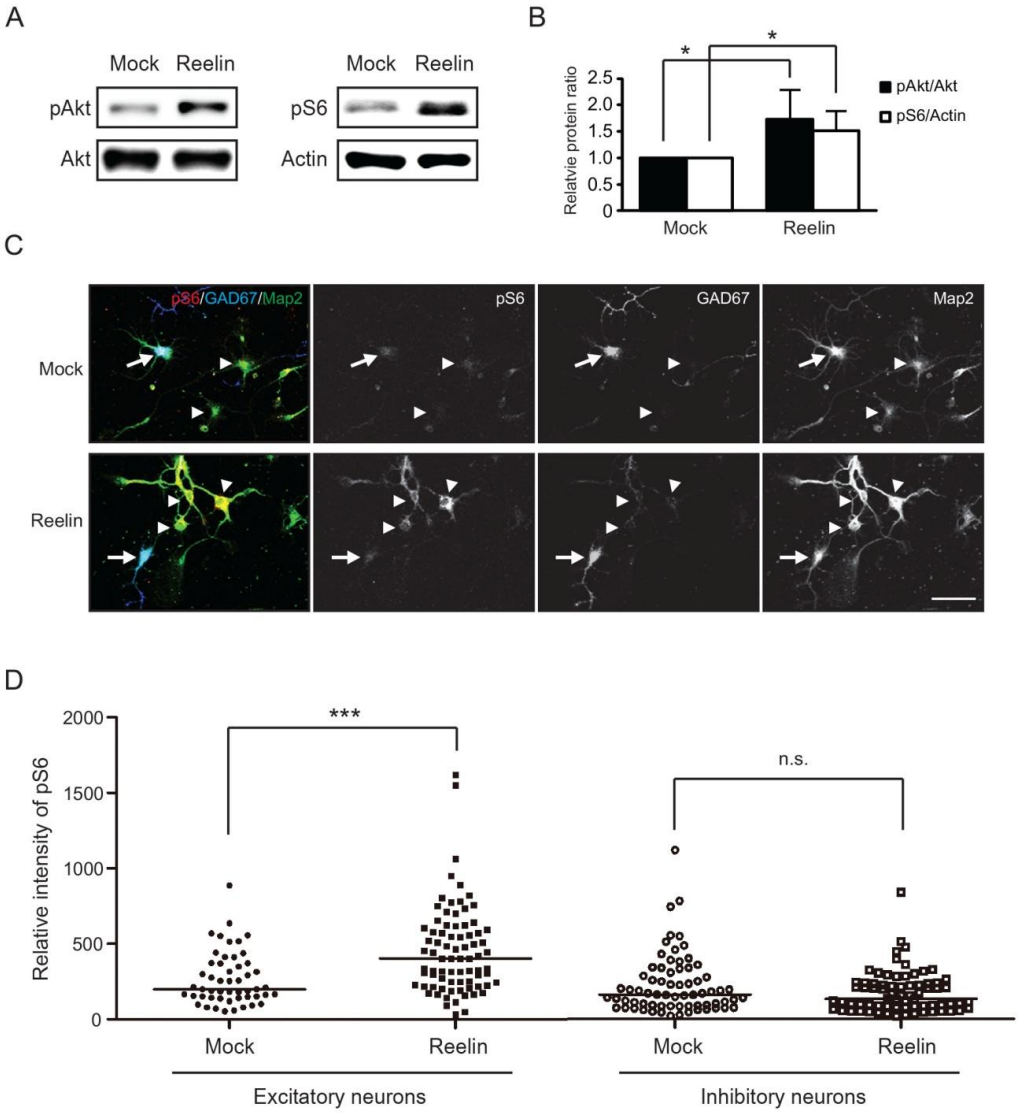

Figure 1. Activation of PI3K/S6 pathway by Reelin in inhibitory neurons.

(A). Five to six days *in vitro* cortical neurons were treated with Reelin for 15-30 minuetes and assayed using western blot analysis. The levels of phospho-Akt and phospho-S6 were significantly increased by Reelin. (B). The results were averaged from six individual experiments and analyzed using one-sample *t*-test. (C). Five to six days *in vitro* hippocampal neurons were imaged by confocal microscope using Map2, GAD67, or phospho-S6 antibodies. Noticeable increase in phospho-S6 levels was shown only in GAD67-negative/Map2-positive excitatory neurons. (D). Quantification and sample distribution of individual neurons in images (number of excitatory neurons=51-78, number of inhibitory neurons=62-74). Quantification was analyzed by Student's t-test. Error bars represent standard deviation (SD). * $p < 0.05$, *** $p < 0.001$ and n.s., nonsignificant. Scale bars, 50 μm.

2. Reelin reduces the area and intensity of inhibitory postsynaptic density.

To study whether Reelin affects the development of inhibitory synapse, we analyzed inhibitory synapse in 11-12 days *in vitro* hippocampal neurons using immunofluorescence and confocal microscopy. We used hippocampal neurons for morphological studies of inhibitory synapse because of their better homogeneity than cortical neurons [3, 19, 22]. Gephyrin, an inhibitory postsynaptic marker, and VGAT, an inhibitory presynaptic marker, were used for measuring the density of inhibitory synapse. The density of Gephyrin- and VGAT-positive inhibitory synapse was not changed 12 hours after Reelin addition (Fig. 2A, 2B). However, the area and intensity of Gephyrin cluster were reduced significantly, whereas those of VGAT cluster were not altered (Fig. 2A, 2B). The altered levels of area and intensity of Gephyrin cluster were 3.07 (3.42) μm^2, 64.98 (70.85)% compared to mock-treated control 4.63 (6.40) μm^2, 117.20 (149.45)%, respectively.

These data suggest that Reelin influences the presynaptic strength of inhibitory synapse without direct activation of PI3K/Akt/S6 pathway in inhibitory neurons.

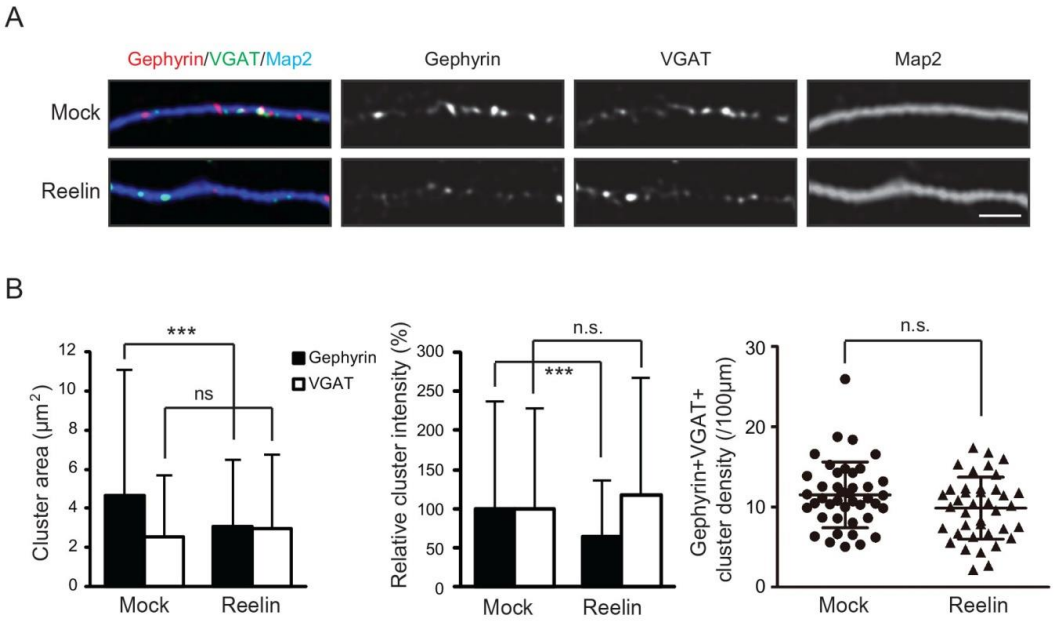

Figure 2 Image analysis area and intensity of inhibitory pre- or post-synaptic density by Reelin.

(A). Eleven to twelve days *in vitro* hippocampal neurons were imaged using immunofluorescence, followed by confocal microscopy in response to Reelin for 12 hrs. Gephyrin or VGAT clusters were seen on Map2-positive neurons. (B). Quantification of synaptic gephyrin or VGAT clusters. The area and relative intensity of Gephyrin cluster were significantly reduced upon Reelin supplementation, while the density was not altered. Also, the area and intensity of VGAT cluster were not changed. Results (number of dendritic fields=40-48) were analyzed using Student *t*-test. Error bars represent standard deviation (SD). *** $p < 0.001$ and n.s., nonsignificant. Scale bars, 5 μm.

3. Expression of inhibitory synapse-related genes by Reelin.

To understand potential molecular mechanisms of Reelin in inhibitory synapse regulation, we analyzed the expression of a series of genes which are involved in inhibitory synapse development in primary cortical neurons. Western blotting analysis showed that expression level of protein including GAD67, GAD65, Gephyrin, and VGAT was not changed 12 hours after Reelin treatment (Fig. 3A, 3B). Also, mRNA level of genes including GAD65, Neuroligin1, Neuroligin2, Semaphorin4D, and FGF7 was not altered (Fig. 3C). However, the quantity of GAD67 transcript was significantly reduced 12 hours of Reelin treatment (Fig. 3C), which might be further investigated in accordance with protein expression.

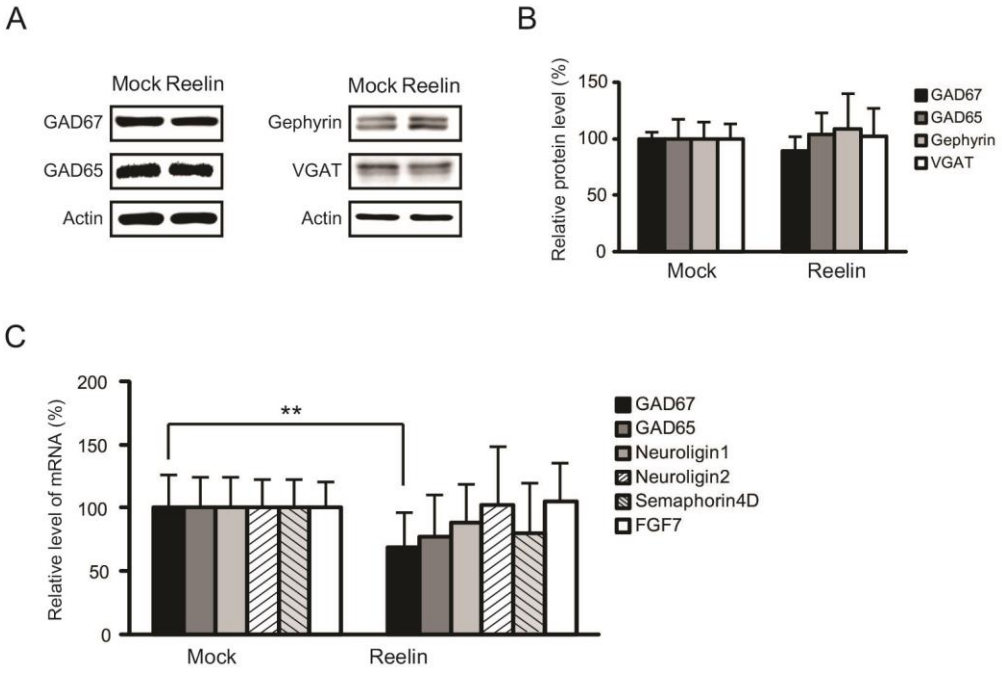

Figure 3. Expression of inhibitory synapse-related genes by Reelin.

(A). Eleven to twelve days *in vitro* cortical neurons were treated with Reelin for 12 hours and assayed using western blot analysis. The expression level of inhibitory neuron-related proteins was not changed upon Reelin treatment for 12 hours. (B). The results were averaged from four individual experiments. (C). mRNA contents were quantified using quantitative real time-PCR and analyzed using Student *t*-test. The level of GAD67 transcript was significantly reduced upon Reelin treatment, but not GAD65, Neuroligin1, Neuroligin2, Semaphorin4D, and FGF7. Quantification was analyzed by Student's t-test. Error bars represent standard deviation (SD). * $p < 0.05$

Ⅳ. DISCUSSION

Although Reelin is a critical protein on the excitatory neuronal migration during corticogenesis, it has appeared not to affect the positioning of inhibitory neurons directly. However, since Reelin has been associated with several processes of neuronal development and function including dendrite elongation, spine formation and synaptic plasticity in postnatal days, it has been questioned about the implication of Reelin in the function of inhibitory neurons. Reelin exerts the several roles through Dab1 and subsequent activation of PI3K/Akt signaling pathway, especially for dendrite and spine development of neurons. Also, a previous study has shown that the adaptor protein, Dab1, is expressed in inhibitory neurons. Yet, it has not been known whether Reelin directly activates the well-known signaling pathway of Reelin via Dab1 in inhibitory neurons, or whether Reelin stimulates another signaling pathway. In this study, we examined the activation of intracellular signaling of individual inhibitory neurons by Reelin using immunofluorescence. Although the activation of S6 protein under PI3K signaling pathway in GABA-positive inhibitory neurons was not influenced by Reelin, the inhibitory synapse was affected, which was shown as a decrease in the intensity and area of Gephyrin clusters without the apparent expression change of inhibitory synapse-related proteins.

It was unexpected that Reelin did not activate S6 protein. It may be because of no activation of PI3K/Akt even in the presence of Dab1, or because of activation of other signaling pathways under PI3K pathway. Due to limited antibodies for immunocytochemistry of phosphorylated Akt or Dab1, it is not easy to determine directly how Reelin exert the roles in inhibitory synapse formation in inhibitory neurons. Moreover,

inhibitory synapses are developed from the contact of axons from several subgroups of inhibitory neurons that are differentially activated according to behavioral states. And, canonically most inhibitory synapses on distal dendrites arise from somatostatin (SOM)-expressing inhibitory neurons [10, 24]. Thus, further immunofluorescence assays need to be done to investigate which subgroups of inhibitory neurons response to Reelin.

Since Reelin has been known to stimulate the formation of dendritic spines for excitatory synapses, it may affect the development of inhibitory synapse without direct activation of inhibitory neurons by Reelin. The interaction between excitatory machinery and inhibitory synapse development has been shown in previous studies that glutamate receptor activities are required for GABAergic synapse development [1, 12, 18, 25]. Specifically, postsynaptic alteration of Gephyrin clusters without presynaptic change of VGAT clusters suggest a possibility that excitatory neurons responding to Reelin regulates inhibitory synapse formation.

In summary, our results demonstrated that Reelin attenuates inhibitory synapse formation not through well-known Reelin signaling pathway, PI3K/Akt/S6, in hippocampal neurons. This study reveals a new function of Reelin in GABAergic inhibitory synapse development and contributes to cellular mechanisms underlying Reelin-associated neurological diseases.

Ⅴ. REFERENCES

1. Aamodt, S.M., Shi, J., Colonnese, M.T., Veras, W., and Constantine-Paton, M. (2000). Chronic NMDA exposure accelerates development of GABAergic inhibition in the superior colliculus. Journal of neurophysiology ***83***, 1580-1591.

2. Alcantara, S., Ruiz, M., D'Arcangelo, G., Ezan, F., de Lecea, L., Curran, T., Sotelo, C., and Soriano, E. (1998). Regional and cellular patterns of reelin mRNA expression in the forebrain of the developing and adult mouse. The Journal of neuroscience : the official journal of the Society for Neuroscience ***18***, 7779-7799.

3. Arimura, N., and Kaibuchi, K. (2007). Neuronal polarity: from extracellular signals to intracellular mechanisms. Nature reviews Neuroscience ***8***, 194-205.

4. Chao, H.T., Chen, H., Samaco, R.C., Xue, M., Chahrour, M., Yoo, J., Neul, J.L., Gong, S., Lu, H.C., Heintz, N., *et al.* (2010). Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. Nature ***468***, 263-269.

5. Cline, H. (2005). Synaptogenesis: a balancing act between excitation and inhibition. Current biology : CB ***15***, R203-205.

6. Costa, E., Chen, Y., Davis, J., Dong, E., Noh, J.S., Tremolizzo, L., Veldic, M., Grayson, D.R., and Guidotti, A. (2002). REELIN and schizophrenia: a disease at the interface of the genome and the epigenome. Molecular interventions ***2***, 47-57.

7. Curley, A.A., Arion, D., Volk, D.W., Asafu-Adjei, J.K., Sampson, A.R., Fish, K.N., and Lewis, D.A. (2011). Cortical deficits of glutamic acid decarboxylase 67 expression in schizophrenia: clinical, protein, and cell type-specific features. The American journal of psychiatry ***168***, 921-929.

8. D'Arcangelo, G., Miao, G.G., Chen, S.C., Soares, H.D., Morgan, J.I., and Curran, T. (1995). A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. Nature ***374***, 719-723.

9. De Rubeis, S., He, X., Goldberg, A.P., Poultney, C.S., Samocha, K., Cicek, A.E., Kou, Y., Liu, L., Fromer, M., Walker, S., *et al.* (2014). Synaptic, transcriptional and chromatin genes disrupted in autism. Nature ***515***, 209-215.

10. Di Cristo, G., Wu, C., Chattopadhyaya, B., Ango, F., Knott, G., Welker, E., Svoboda, K., and Huang, Z.J. (2004). Subcellular domain-restricted GABAergic innervation in primary visual cortex in the absence of sensory and thalamic inputs. Nature neuroscience ***7***, 1184-1186.

11. Fatemi, S.H., Earle, J.A., and McMenomy, T. (2000). Reduction in Reelin immunoreactivity in hippocampus of subjects with schizophrenia, bipolar disorder and major depression. Molecular psychiatry ***5***, 654-663, 571.

12. Hartman, K.N., Pal, S.K., Burrone, J., and Murthy, V.N. (2006). Activity-dependent regulation of inhibitory synaptic transmission in hippocampal neurons. Nature neuroscience ***9***, 642-649.

13. Jossin, Y., and Goffinet, A.M. (2007). Reelin signals through phosphatidylinositol 3-kinase and Akt to control cortical development and through mTor to regulate dendritic growth. Molecular and cellular biology ***27***, 7113-7124.

14. Rubenstein, J.L. (2010). Three hypotheses for de Puskarjov, M. (2014). GABA actions and ionic plasticity in epilepsy. Current opinion in neurobiology ***26***, 34-41.

15. Lee, G.H., Chhangawala, Z., von Daake, S., Savas, J.N., Yates, J.R., 3rd, Comoletti, D., and D'Arcangelo, G. (2014). Reelin induces Erk1/2 signaling in cortical neurons through a non-canonical pathway. The Journal of biological chemistry ***289***, 20307-20317.

16. Lee, G.H., and D'Arcangelo, G. (2016). New Insights into Reelin-Mediated Signaling Pathways. Frontiers in cellular neuroscience ***10***, 122.

17. Lisman, J. (2012). Excitation, inhibition, local oscillations, or large-scale loops: what causes the symptoms of schizophrenia? Current opinion in neurobiology *22*, 537-544.

18. Marty, S., Wehrle, R., and Sotelo, C. (2000). Neuronal activity and brain-derived neurotrophic factor regulate the density of inhibitory synapses in organotypic slice cultures of postnatal hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience ***20***, 8087-8095.

19. Nelson, S.B., Sugino, K., and Hempel, C.M. (2006). The problem of neuronal cell types: a physiological genomics approach. Trends in neurosciences ***29***, 339-345.

20. Nikolaeva, I., Kazdoba, T.M., Crowell, B., and D'Arcangelo, G. (2017). Differential roles for Akt and mTORC1 in the hypertrophy of Pten mutant neurons, a cellular model of brain overgrowth disorders. Neuroscience ***354***, 196-207.

21. Niu, S., Renfro, A., Quattrocchi, C.C., Sheldon, M., and D'Arcangelo, G. (2004). Reelin promotes hippocampal dendrite development through the VLDLR/ApoER2-Dab1 pathway. Neuron ***41***, 71-84.

22. Niu, S., Yabut, O., and D'Arcangelo, G. (2008). The Reelin signaling pathway promotes dendritic spine development in hippocampal neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience ***28***, 10339-10348.

23. Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. Nucleic acids research ***29***, e45.

24. Pla, R., Borrell, V., Flames, N., and Marin, O. (2006). Layer acquisition by cortical GABAergic interneurons is independent of Reelin signaling. The Journal of neuroscience : the official journal of the Society for Neuroscience ***26***, 6924-6934.

25. Rosato-Siri, M., Grandolfo, M., and Ballerini, L. (2002). Activity-dependent modulation of GABAergic synapses in developing rat spinal networks in vitro. The European journal of neuroscience ***16***, 2123-2135.

26. Rubenstein, J.L. (2010). Three hypotheses for developmental defects that may underlie some forms of autism spectrum disorder. Current opinion in neurology ***23***, 118-123.

27. Telese, F., Ma, Q., Perez, P.M., Notani, D., Oh, S., Li, W., Comoletti, D., Ohgi, K.A., Taylor, H., and Rosenfeld, M.G. (2015). LRP8-Reelin-Regulated Neuronal Enhancer Signature Underlying Learning and Memory Formation. Neuron ***86***, 696-710.

28. Trotter, J., Lee, G.H., Kazdoba, T.M., Crowell, B., Domogauer, J., Mahoney, H.M., Franco, S.J., Muller, U., Weeber, E.J., and D'Arcangelo, G. (2013). Dab1 is required for synaptic plasticity and associative learning. The Journal of neuroscience : the official journal of the Society for Neuroscience ***33***, 15652-15668.

29. Yabut, O., Renfro, A., Niu, S., Swann, J.W., Marin, O., and D'Arcangelo, G. (2007). Abnormal laminar position and dendrite development of interneurons in the reeler forebrain. Brain research ***1140***, 75-83.