ISSN (Print) 1225-9918
ISSN (Online) 2287-3406
Journal of Life Science 2014 Vol. 24. No. 1. 14~19 DOI : http://dx.doi.org/10.5352/JLS.2014.24.1.14

PtdIns(3,5)P_2 5-phosphatase Fig4 Interacts with Kinesin Superfamily 5A (KIF5A)

Won Hee Jang and Dae-Hyun Seog*

Departments of Biochemistry, College of Medicine and UHRC, Inje University, Busan 614-735, Korea

Received November 28, 2013 / Revised December 19, 2013 / Accepted December 19, 2013

Kinesin-1 consists of two heavy chains (KHCs), also called KIF5s, and two light chains (KLCs) that form a heterotetrameric complex. Here, we demonstrate the binding of a neuronal KHC, KIF5A, to the carboxyl (C)-terminal tail region of Fig4 (also known as Sac3), a phosphatase that removes the 5-phosphate from phosphatidylinositol-3,5-bisphosphate (PtdIns(3,5)P_2). Fig4 bound to the C-terminal region of KIF5A but not to other KHCs (KIF5B and KIF5C) and KLC1 in yeast two-hybrid assays. The interaction was further confirmed in a glutathione *S*-transferase pull-down assay and by co-immunoprecipitation. Anti-KIF5A antibody co-immunoprecipitated Fig4 with KIF5A from mouse brain extracts. These results suggest that kinesin-1 could transport the Fig4-associated protein complex or cargo in cells.

Key words : Adaptor protein, Fig4, protein-protein interaction, kinesin-1, microtubule motors, Sac3

Introduction

Compared with other cells, neurons with long neurites have a well-developed transport system. Intracellular transport mechanism is controlled by microtubule-dependent motor proteins [5]. Kinesin-1 is a motor protein that utilizes ATP hydrolysis to drive the intracellular transport along microtubules and has been reported to transport various types of cargoes, including organelles, synaptic vesicle precursors, neurotransmitter receptors, cell signaling molecules, and mRNAs in the neurons [5, 10]. Kinesin-1 is a heterotetramer composed of two kinesin heavy chains (KHCs, also called KIF5s) and two kinesin light chains (KLCs) [10]. KHC has a motor domain that hydrolyzes ATP. KLC is involved in modulation of KHC motor activity [5]. KHC has specific cargo-binding regions and KLC-binding domains in its carboxyl (C)-terminus [11]. There are three KHCs (KIF5A, KIF5B, and KIF5C), which are encoded by different genes and possess a similar motor domain containing a microtubule-binding motif in the amino (N)-terminus and a different carboxyl (C)-terminus. KIF5B appears to be ubiquitously expressed, whereas KIF5A and KIF5C appear to be expressed in neuron [14].

*Corresponding author
Tel : +82-51-890-6974, Fax : +82-51-894-5801
E-mail : daehyun@inje.ac.kr
This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Using the many new methods makes it possible to identify the binding proteins of kinesin-1 [11]. KIF5s-binding proteins include glutamate receptor-interacting protein 1 (GRIP1) [21], mRNA/protein (mRNP) complex [13], SNAP25 [6], syntabulin [22], β-dystrobrevin [11], DISC1 [11], and GABARAP [18]. The proteins that bind to the tetratricopeptide repeat (TRP) domain of KLC have also been identified. These include c-jun NH_2-terminal kinase (JNK)-interacting proteins (JIPs) [4] and amyloid-β precursor protein (APP) [17].

To investigate *in vivo* functions of KIF5s, *kif5* knockout (KO) mice were generated. *Kif5b*-KO mice are embryonic lethal and show abnormal localization of mitochondria [24]. *Kif5c*-KO mice are normal in their appearance but show a smaller brain size [14]. *Kif5a*-KO mice are neonatal lethal but show no apparent histological abnormalities in their brains except that the nuclei and cell bodies of spinal cord motor neurons appear to be larger than the wild-type (WT) [25]. More than 75% of conditional *Kif5a*-KO mice undergo seizures and die within 3 weeks [25]. KIF5A has been suggested to be important for the viability of neurons [25]. KIF5A mutations have been found to cause SPG10, a rare form of hereditary spastic paraplegia (SPG) in human [19]. However, despite increasing number of reports for cargoes carried by kinesin-1, KIF5A-specific cargo or binding protein(s) capable of explaining these phenotypes are still unknown. Thus, we searched for KIF5A-specific binding partners that bind to the C-terminal region of KIF5A and found an interaction with Fig4, a phosphatidylinositol-3,5-bisphosphate (PtdIns(3,5)P_2) 5-phosphatase [12]. The KIF5A and Fig4 interaction suggests

that kinesin-1 may transport Fig4-associated cargo in cells.

Materials and Methods

Cloning of full-length mouse Fig4 cDNA and other constructs

Full-length mouse Fig4 (accession NM_133999), was amplified by polymerase chain reaction (PCR) from Marathon-Ready™cDNA library (Clontech, Palo Alto, CA, USA) and cloned into pGEM T-easy vector (Promega Corp, Madison, WI, USA). The cDNA fragments corresponding to the C-terminal regions of mouse KIF5s and KLC1 were also amplified and cloned into pGEM T-easy vector (Promega). The resulting recombinant plasmid was then cut with *EcoR*I and *Xho*I and the insert was subcloned into pLexA (Clontech), pJG4-5 (Clontech), and pET41a (Novagen, San Diego, CA, USA).

Screening of KIF5A-binding proteins by yeast two-hybrid system

The Matchmaker LexA two-hybrid system was used for screening according to the manufacturer's manual (Clontech). In brief, yeast strain EGY48 carrying the p8op-*lacZ* reporter plasmid was transformed with pLexA-KIF5A. The yeast cells were subsequently transformed with the mouse brain cDNA library and grown on synthetic dextrose (SD) plates supplemented with glucose but with no histidine, tryptophan, or uracil (SD/-His/-Trp/-Ura). The selection of positive clones was performed on an SD/-His/-Trp/-Ura/-Leu plate containing galactose, raffinose, X-gal, and BU salts. Plasmids from positive clones were analyzed by digestion with *EcoR*I and *Xho*I. Unique inserts were sequenced and protein sequence analysis was performed with the BLAST algorithm at the National Center for Biotechnology Information (NCBI). Sequence-verified clones were tested again for interaction with the bait in yeast by retransformation.

β-Galactosidase activity in liquid cultures of yeast

The β-galactosidase activity of yeast was assayed as described previously [16, 23]. Mid-log phase yeast cells were collected and permeabilized with 0.1% sodium dodecyl sulfate (SDS) and chloroform. An excess amount of o-nitrophenyl-β-D-galactoside (ONPG) was added to yeast lysate. The mixture was incubated at 30°C and then the reaction was stopped by increasing pH to 11 by the addition of 1 M Na_2CO_3. The formation of the reaction product, o-nitrophenol, was determined by measuring absorbance at 420 nm on a spectrophotometer and normalizing for the reaction time. The units of enzyme activity were calculated by the following equation: units=$1000\times[(OD_{420} - 1.75\times OD_{550})]/(\text{reaction time}\times\text{culture volume}\times OD_{600})$. All experiments were independently performed at least three times [2].

Glutathione S-transferase (GST) pull-down assays

The cDNA fragments of Fig4 were cloned into pET41a. The recombinant GST-Fig4 fusion proteins were expressed in bacterial strain BL21 GOLD (Stratagene, La Jolla CA, USA) after induction with 0.5 mM isopropyl thio-β-D-galactopyranoside (IPTG) for 2 hr. The fusion proteins were purified using glutathione-agarose beads (Sigma-Aldrich) according to the manufacturer's protocol. The mouse brain S2 fraction was incubated overnight at 4°C with the GST fusion protein-coupled glutathione beads. The beads were pelleted by centrifugation, washed three times with the extraction buffer (1% Triton X-100 in PBS containing 10 µg/ml each aprotinin, leupeptin, and pepstatin and 1 µM phenylmethanesulfonyl fluoride), and once with PBS. The bound proteins were eluted from the glutathione beads with 100 µl of Laemmli's loading buffer. The pulled-down proteins were analyzed by immunoblotting with anti-KIF5A, anti-KIF5B, anti-KIF5C, and anti-KLC1 antibodies [14].

Co-immunoprecipitation

Mouse brains were homogenized in ice-cold homogenization buffer (0.32 M sucrose, 4 mM HEPES, pH 7.3) supplemented with protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). For immunoprecipitation, mouse brain lysate was diluted in the same volume of 2X binding buffer (50 mM HEPES, 200 mM KCl, 0.2% Triton X-100, pH 7.0) and incubated with anti-KIF5A antibody, anti-KIF5B antibody, anti-KIF5C antibody [14], or preimmune rabbit serum overnight at 4°C, followed by precipitation with protein-A Sepharose (Amersham Pharmacia, Piscataway, NJ, USA). The beads were collected by brief centrifugation and washed three times with TBS-T (20 mM Tris-HCl, pH 7.5, 0.2 M NaCl, 0.1% Tween 20). The washed beads were resuspended with Laemmli's loading buffer and the proteins were eluted and denatured by boiling for 2 minutes and then separated by SDS-PAGE. The proteins were transferred from the gel to a nitrocellulose membrane and incubated with anti-Fig4 antibody (Abcam, Cambridge, MA, USA).

Results

Identification of KIF5A interacting protein by yeast two-hybrid screening

KIF5A has the C-terminal region of 73 amino acids that shows no homology with KIF5B or KIF5C [14]. To identify KIF5A interacting proteins, we screened a mouse brain cDNA library by yeast two-hybrid system using the C-terminal 73 amino acids (aa 955-1027) of KIF5A as bait. From 8 $\times 10^6$ colonies screened, we identified a clone that encodes Fig4 as a binding partner for KIF5A. This clone possessed a cDNA fragment corresponding to the C-terminal region (aa 795-907) of Fig4 (Fig. 1A). Fig4 was isolated as a vacuolar-associated PtdIns(3,5)P_2 5-phosphatase related to the suppressor of actin (Sac) 1 PtdInsP phosphatase [9, 20]. To determine the minimal binding domain of Fig4 that is required for the interaction with KIF5A, we constructed several deletion mutants of Fig4. Yeast two-hybrid assays showed that the minimal domain required for binding was located in the C-terminal tail region of Fig4 (Fig. 1B). Next, we determined the minimal binding domain of KIF5A. KIF5A is composed of motor domain, neck domain, stalk domain, and KLC binding domain [14]. We constructed several deletion mutants of KIF5A and tested for the interaction with Fig4. The interaction with Fig4 was dependent on the C-terminal region of KIF5A (Fig. 1C). Together, these results show that the interaction between KIF5A and Fig4 is mediated through their C-terminal regions.

KIF5A consists of 1027 amino acids and shows high sequence homology to KIF5B or KIF5C (60% identical) [14]. Next we analyzed whether Fig4 interacts with KIF5B, KIF5C, and KLC1 subunits of kinesin-1. As shown in Fig. 2A, there was no detectable binding of Fig4 with KIF5B, KIF5C, and KLC1. A quantitative β-galactosidase assay showed that Fig4 directly bound to KIF5A but not to other KIF5s and KLC1 (Fig. 2B). This result was not surprising in view of the fact that KIF5A, KIF5B, and KIF5C have no homology in their C-terminal regions [14]. These data indicate that the interaction of KIF5A with Fig4 is specific and the C-terminal 73 amino acids of KIF5A were sufficient for the interaction.

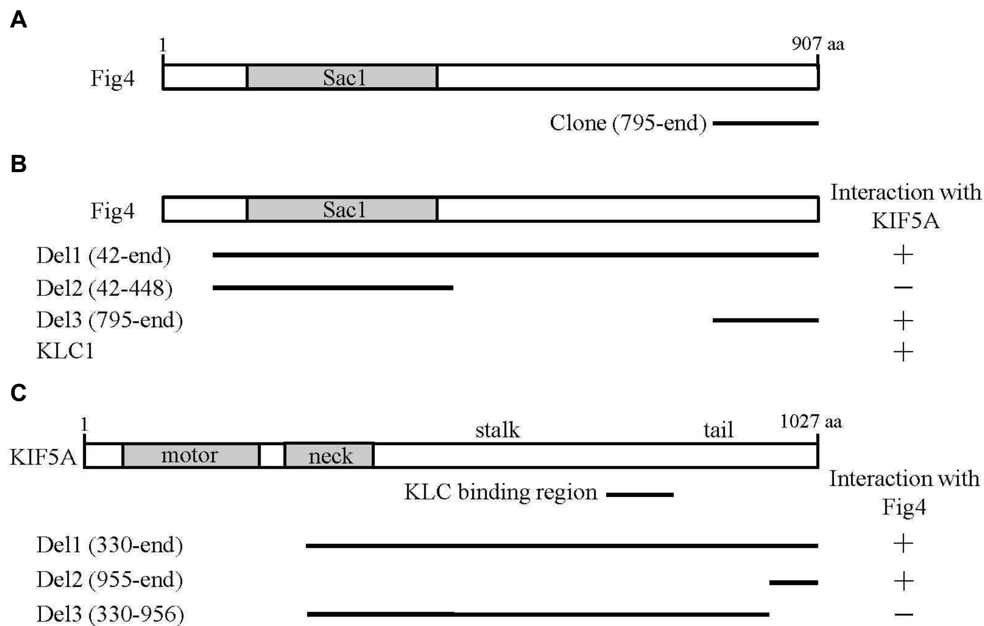

Fig. 1. Identification of KIF5A interacting proteins by yeast two-hybrid screening. (A) The domain structure of Fig4 illustrating that the positive clone corresponds to the C-terminal region of Fig4. The putative Sac1 domain is indicated in gray. (B) To determine minimal KIF5A binding region and binding specificity, several truncated forms of Fig4 were constructed by PCR and tested in the yeast two-hybrid assay for interaction with KIF5A. KLC1 construct was used as a positive control for KIF5A binding. +, interaction with KIF5A; -, no interaction with KIF5A. (C) The C-terminal region of KIF5A mediates the interaction with Fig4. KIF5A has a motor domain and a neck domain indicated in gray. Several truncated forms of KIF5A were constructed by PCR and tested in the yeast two-hybrid assay for interaction with Fig4. +, interaction with Fig4; -, no interaction with Fig4. aa, the amino acid residue number.

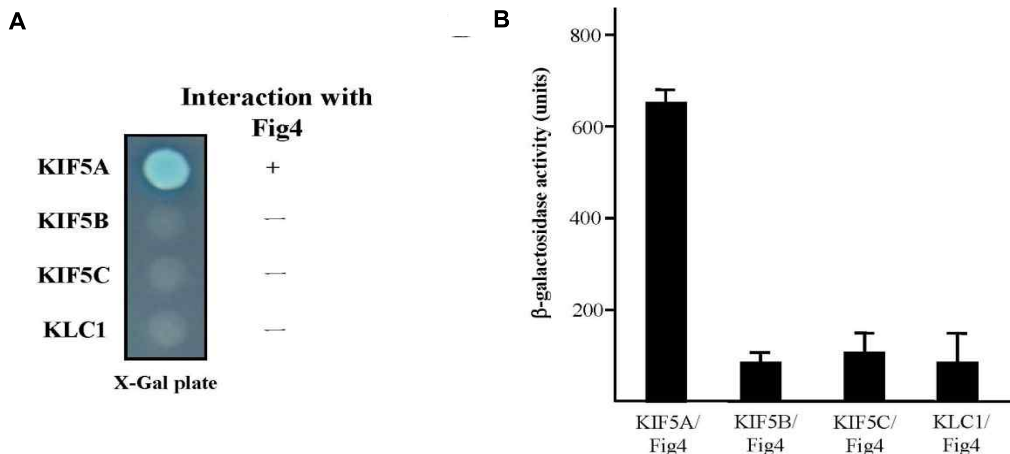

Fig. 2. Interaction between KIFs and Fig4. (A) The C-terminal regions of each KIF5s and KLC1 were fused to the pLexA DNA binding domain. Fig4 specifically interacted with KIF5A but not with KIF5B, KIF5C, and KLC1. +, interaction; -, no interaction. (B) The strength of interactions between KIF5s or KLC1 and Fig4 was examined quantitatively using β - galactosidase activity in yeast two-hybrid reporter assay.

Fig4 is associated with kinesin-1

Kinesin-1 is a heterotetramer made of two KIF5s and two KLCs [10, 14]. Previous immunoprecipitation experiments revealed that heterodimers as well as homodimers of KIF5s may constitute kinesin-1. KIF5A can form heterodimers with KIF5B or KIF5C [14]. Therefore, we next determined whether Fig4 precipitates not only KIF5A but also other kinesin-1 subunits at the protein level using GST pull-down experiments. Recombinant GST-Fig4 fusion proteins were expressed in *E. coli*. The purified GST fusion proteins were allowed to interact with mouse brain lysates. Immunoblotting analyses revealed that KIF5A interacted with GST-Fig4, but not to GST-Fig4-Sac1 domain. KIF5B, KIF5C, and KLC1, which interact with the KIF5A [14], were also efficiently precipitated by GST-Fig4 (Fig. 3A). This result indicates that Fig4 interacts with kinesin-1 complex.

The association of Fig4 with kinesin-1 was further examined by co-immunoprecipitation assay. Lysates from mouse brain were incubated with anti-KIF5A antibody, anti-KIF5B antibody, or anti-KIF5C antibody. Protein A-Separose beads selectively precipitated the immuno-complexes, which were then subsequently separated by SDS-PAGE and immuno-

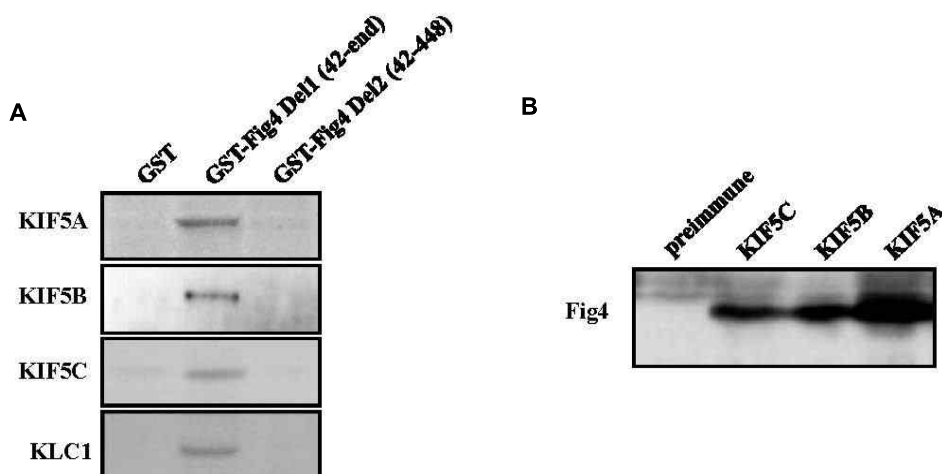

Fig. 3. Association of kinesin-1 with Fig4 in the GST pull-down assay and co-immunoprecipitation. (A) Proteins in the mouse brain lysate were allowed to bind to GST alone or GST-Fig4 fusion proteins. The elution fractions were resolved by SDS-PAGE and analyzed by immuoblotting using anti-KIF5A, KIF5B, KIF5C, or KLC antibodies. (B) Mouse brain lysates were immunoprecipitated with anti-KIF5A, KIF5B, or KIF5C antibodies, or preimmune serum, and then the precipitates were immunoblotted with anti-Fig4 antibody.

blotted with anti-Fig4 antibody. As shown in Fig. 3B, anti-KIF5A, anti-KIF5B, and anti-KIF3A antibodies efficiently precipitated Fig4. This data confirms that Fig4 is associated with kinesin-1.

Discussion

In this study, we have shown that KIF5A interacts with the PtdIns(3,5)P_2 5-phosphatase Fig4. Using the C-terminal 73 amino acids of KIF5A as bait, we identified Fig4 in yeast two-hybrid screening. We found that KIF5A interacts with the C-terminal region of Fig4. Additionally, using GST pull-down and co-immunoprecipitation assays, we confirmed that Fig4 interacts with KIF5A at the protein level. Moreover, we showed that Fig4 can be co-precipitated with kinesin-1 complex. These results suggest that kinesin-1 may transport Fig4 and/or cargo associated with Fig4 through the interaction between KIF5A and Fig4.

Lipids including phosphoinositides are implicated in signal transduction, organelle trafficking, and intracellular transport [12]. PtdIns(3,5)P_2 is one of seven phosphoinositides and a low-abundant species that is mainly found in endolysosomes [12, 20]. PtdIns(3,5)P_2 dysfunction is implicated in some human neurodegenerative diseases such as Charcot-Marie-Tooth Type 4J (CMT4J) disease and amyotrophic lateral sclerosis [7, 15]. Patients with CMT4J, which is caused by loss-of-function mutation of *FIG4* gene, exhibit neurodegeneration in the central and peripheral nervous systems, affecting sensory and motor neurons [7, 26]. CMT4J cells possess enlarged endolysosomes and lysosomes [7]. It is unclear how Fig4 deficiency causes abnormal endolysosomes and lysosomes. In mammals, Fig4 mainly localizes to lysosomes [12, 20]. PtdIns(3,5)P_2 is known to relate to the endolysosome morphology, trafficking, and acidification [12], which, therefore, could be modulated by Fig4-mediated PtdIns(3,5)P_2 turnover. Fig4 binds directly to Vac14, which is necessary for PtdIns(3,5)P_2 synthesis [3, 8, 12]. Interestingly, Fig4-Vac14 complex interacts with PtdIns(3)P5-kinase Fab1. Fab1 localizes to endolysosomes or lysosomes [8]. Vac14 is necessary for the interaction between Fig4 and Fab1, indicating that Vac14 is an adaptor protein for formation of Fig4-Vac14-Fab1 complex [1]. The Fig4-Vac14-Fab1 complex may regulate PtdIns(3,5)P_2 synthesis and turn over on endolysosomes or lysosomes.

As described in this study, KIF5A directly interacted with Fig4. Although we did not identify Fig4-associated cargo, this data suggested that the interaction between KIF5A and Fig4 may link kinesin-1 to endolysosomes or lysosomes through Fig4-Vac14-Fab1 complex. In conclusion, we favor the model that Fig4, involved in regulation of PtdIns(3,5)P_2, may also play a role as an adaptor protein in the intracellular transport of endolysosomes or lysosomes by kinesin-1.

Acknowledgments

This research was supported by Basic Science Research Program though the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0021296).

References

1. Alghamdi, T. A., Ho, C. Y., Mrakovic, A., Taylor, D., Mao, D. and Botelho, R. J. 2013. Vac14 protein multimerization is a prerequisite step for Fab1 protein complex assembly and function. *J Biol Chem* **288**, 9363-9372.
2. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. 1998. *Current Protocols in Molecular Biology*. John Wiley & Sons.
3. Bonangelino, C. J. and Weisman, L. S. 2002. Osmotic stress-induced increase of phosphatidylinositol 3,5-bisphosphate requires Vac14p, an activator of the lipid kinase Fab1p. *J Cell Biol* **156**, 1015-1028.
4. Bowman, A. B., Kamal, A., Ritchings, B. W., Philp, A. V., McGrail, M., Gindhart, J. G. and Goldstein, L. S. 2000. Kinesin-dependent axonal transport is mediated by the sunday driver (SYD) protein. *Cell* **103**, 583-594.
5. Brady, S. T. 1985. A novel brain ATPase with properties expected for the fast axonal transport motor. *Nature* **317**, 73-75.
6. Cai, Q., Pan, P. Y. and Sheng, Z. H. 2007. Syntabulin-kinesin-1 family member 5B-mediated axonal transport contributes to activity-dependent presynaptic assembly. *J Neurosci* **27**, 7284-7296.
7. Chow, C. Y., Zhang, Y., Dowling, J. J., Jin, N., Adamska, M., Shiga, K., Szigeti, K., Shy, M. E., Li, J., Zhang, X., Lupski, J. R., Weisman, L. S. and Meisler, M, H. 2007. Mutation of FIG4 causes neurodegeneration in the pale tremor mouse and patients with CMT4J. *Nature* **448**, 68-72.
8. Dove, S. K., McEwen, R. K., Mayes, A., Hughes, D. C., Beggs, J. D. and Michell, R. H. 2002. Vac14 controls PtdIns(3,5)P(2) synthesis and Fab1-dependent protein trafficking to the multivesicular body. *Curr Biol* **12**, 885-893.
9. Gary, J. D., Sato, T. K., Stefan, C. J., Bonangelino, C. J., Weisman, L. S. and Emr, S. D. 2002. Regulation of Fab1 phosphatidylinositol 3-phosphate 5-kinase pathway by Vac7 protein and Fig4, a polyphosphoinositide phosphatase family member. *Mol Biol Cell* **13**, 1238-1251.
10. Hirokawa, N. 1998. Kinesin and dynein superfamily pro-

teins and the mechanism of organelle transport. *Science* **279**, 519-526.
11. Hirokawa, N., Niwa, S. and Tanaka, Y. 2010. Molecular motors in neurons: transport mechanisms and roles in brain function, development, and disease. *Neuron* **68**, 610-638.
12. Ho, C. Y., Alghamdi, T. A. and Botelho, R. J. 2012. Phosphatidylinositol-3,5-bisphosphate: no longer the poor PIP2. *Traffic* **13**, 1-8.
13. Kanai, Y., Dohmae, N. and Hirokawa, N. 2004. Kinesin transports RNA: isolation and characterization of an RNA-transporting granule. *Neuron* **43**, 513-525.
14. Kanai, Y., Okada, Y., Tanaka, Y., Harada, A., Terada, S. and Hirokawa, N. 2000. KIF5C, a novel neuronal kinesin enriched in motor neurons. *J Neurosci* **20**, 6374-6384.
15. Katona, I., Zhang, X., Bai, Y., Shy, M. E., Guo, J., Yan, Q., Hatfield, J., Kupsky, W. J. and Li, J. 2011. Distinct pathogenic processes between Fig4-deficient motor and sensory neurons. *Eur J Neurosci* **33**, 1401-1410.
16. Kim, S. J., Lee, C. H., Park, H. Y., Yea, S. S., Jang, W. H., Lee, S. K., Park, Y. H., Cha, O. S., Moon, I. S. and Seog, D. H. 2007. JSAP1 interacts with kinesin light chain 1 through conserved binding segments. *J Life Sci* **17**, 889-895.
17. Muresan, Z. and Muresan, V. 2005. Coordinated transport of phosphorylated amyloid-beta precursor protein and c-Jun NH2-terminal kinase-interacting protein-1. *J Cell Biol* **171**, 615-625.
18. Nakajima, K., Yin, X., Takei, Y., Seog, D. H., Homma, N. and Hirokawa, N. 2012. Molecular motor KIF5A is essential for GABA(A) receptor transport, and KIF5A deletion causes epilepsy. *Neuron* **76**, 945-961.
19. Reid, E., Kloos, M., Ashley-Koch, A., Hughes, L., Bevan, S., Svenson, I. K., Graham, F. L., Gaskell, P. C., Dearlove, A., Pericak-Vance, M. A., Rubinsztein, D. C. and Marchuk, D. A. 2002. A kinesin heavy chain (KIF5A) mutation in hereditary spastic paraplegia (SPG10). *Am J Hum Genet* **71**, 1189-1194.
20. Sbrissa, D., Ikonomov, O. C., Fu, Z., Ijuin, T., Gruenberg, J., Takenawa, T. and Shisheva, A. 2007. Core protein machinery for mammalian phosphatidylinositol 3,5-bisphosphate synthesis and turnover that regulates the progression of endosomal transport. Novel Sac phosphatase joins the ArPIKfyve-PIKfyve complex. *J Biol Chem* **282**, 23878-23891.
21. Setou, M., Seog, D. H., Tanaka, Y., Kanai, Y., Takei, Y., Kawagishi, M. and Hirokawa, N. 2002. Glutamate-receptor-interacting protein GRIP1 directly steers kinesin to dendrites. *Nature* **417**, 83-87.
22. Su, Q., Cai, Q., Gerwin, C., Smith, C. L. and Sheng, Z. H. 2004. Syntabulin is a microtubule-associated protein implicated in syntaxin transport in neurons. *Nat Cell Biol* **6**, 941-953.
23. Takeda, S., Yamazaki, H., Seog, D. H., Kanai, Y., Terada, S. and Hirokawa, N. 2000. Kinesin superfamily protein 3 (KIF3) motor transports fodrin-associating vesicles important for neurite building. *J Cell Biol* **148**, 1255-1265.
24. Tanaka, Y., Kanai, Y., Okada, Y., Nonaka, S., Takeda, S., Harada, A. and Hirokawa, N. 1998. Targeted disruption of mouse conventional kinesin heavy chain, kif5B, results in abnormal perinuclear clustering of mitochondria. *Cell* **93**, 1147-1158.
25. Xia, C. H., Roberts, E. A., Her, L. S., Liu, X., Williams, D. S., Cleveland, D. W. and Goldstein, L. S. 2003. Abnormal neurofilament transport caused by targeted disruption of neuronal kinesin heavy chain KIF5A. *J Cell Biol* **161**, 55-66.
26. Zhang, X., Chow, C. Y., Sahenk, Z., Shy, M. E., Meisler, M. H. and Li, J. 2008. Mutation of FIG4 causes a rapidly progressive, asymmetric neuronal degeneration. *Brain* **131**, 1990-2001.

초록 : PI(3,5)P_2 5-phosphatase Fig4와 Kinesin superfamily 5A (KIF5A)의 결합

장원희 · 석대현*
(인제대학교 의과대학 생화학교실)

Kinesin-1은 2개의 장쇄(KHCs, 또는 KIF5s)와 2개의 단쇄(KLCs)가 결합한 복합체로 되어 있다. 본 연구에서 효모 two-hybrid system을 이용하여 중추신경계의 신경세포에서 주로 발현되는 KIF5A와 결합하는 단백질을 탐색한 결과 phosphatidylinositol-3,5-bisphosphate (PI(3,5)P_2)의 5번 위치 인산을 제거하는 탈인산화효소 Fig4 (Sac3)를 분리하였다. KIF5A는 Fig4의 C-말단과 결합함을 효모 two-hybrid assay로 확인하였다. Fig4는 KIF5A의 C-말단과 결합하지만, 두 개의 다른 장쇄인 KIF5B와 KIF5C 그리고 KLC1와는 결합하지 않았다. 단백질 간 결합을 glutathione *S*-transferase pull-down assay와 공동면역침강으로 추가 검증하였다. 생쥐의 뇌 파쇄액을 KIF5A 항체로 면역 침강한 결과 Fig4가 같이 침강하였다. 이러한 결과들은 kinesin-1이 Fig4와 결합한 단백질 복합체 혹은 운반체를 세포 내에서 운반함을 시사한다.