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The heterotrimeric kinesin-2 family member KIF3A directly binds to disabled-1 (Dab1)

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The heterotrimeric molecular motor kinesin-2 is involved in the microtubule-dependent transport of intracellular cargo. It consists of two distinct motor subunits (KIF3A, and KIF3B) and a non-motor subunit, kinesin-associated protein 3 (KAP3). The cargo-binding domain (CBD) at the carboxyl (C)-terminus of KIF3s plays an important role in the interaction with several different binding proteins. To identify the binding proteins for heterotrimeric kinesin-2, we performed a yeast two-hybrid screen and found a new interaction with Disables-1 (Dab1), the intracellular adaptor protein of reelin receptors. Dab1 bound to the CBD of KIF3A, but did not interact with the C-terminal domain of KIF3B, KIF5B, KIF17 or KAP3. The phosphotyrosine binding (PTB) domain-containing region of Dab1 is essential for the interaction with KIF3A. KIF3A interacted with GST-Dab1, and GST-CaMKIIα, but did not interact with GST-apolipoprotein E receptor 2 (ApoER2)-C or with GST alone. When co-expressed in HEK-293T cells, Dab1 co-precipitated with KIF3A, but not with KIF5B. Dab1 and KIF3A were co-localized in cultured cells. We also identified deduced cell surface expression of ApoER2 in KIF3A dominant-negative cells. These results suggest that the KIF3A plays a role in the intracellular trafficking of ApoER2 to the cell surface. [BMB Reports 2024; 57(10): 447-452]

INTRODUCTION

Intracellular trafficking of protein complexes, various protein-containing vesicles, and many organelles contributes to the physiological functions of many cell types. Kinesin and cytoplasmic dynein have been identified as the two major microtubule-dependent motor proteins (1). Kinesins are motor proteins that move in the plus-end direction (2). They play an important role in transporting various cargoes such as vesicles, organelles (3, 4). Kinesin-2 is a member of the kinesin superfamily motor proteins (KIFs) (2, 5). There are two subfamilies found in mammalian cells: heterotrimeric motor and homodimeric motor (5). Heterotrimeric kinesin-2 is composed of two different motor proteins (KIF3A, and KIF3B), which mediate plus-end directed microtubule-dependent cargo transport, and a non-motor subunit, kinesin-associated protein 3 (KAP3) (6). KIF3A and KIF3B have different tissue expression patterns. KIF3A is expressed in a wide range of tissues, including brain, heart, liver, and adipose tissue. On the other hand, KIF3B is highly expressed in brain and testis, and less so in other tissues such as heart and lung (5, 6).

Heterotrimeric kinesin-2 plays an important role in the intracellular transport of many different vesicles and organelles, including N-cadherin, and fodrin-associated vesicles (5, 7, 8). How heterotrimeric kinesin-2 can bind to different cargoes needs to be investigated. In some cases, the cargoes bind adaptor proteins. These proteins mediate the attachment of heterotrimeric kinesin-2 to the cargo (4, 8). KIF3A downregulates the Wnt/β-catenin signaling pathway by binding to β-arrestin. β-arrestin acts as an adaptor protein to form a complex with DVL2 and axin to stabilize β-catenin (9). In addition, protein kinase A (PKA) and calcium/calmodulin-dependent protein kinase IIα (CaMKIIα) were identified as regulatory proteins that regulate the binding between the heterotrimeric kinesin-2 and N-cadherin (8). Phosphorylation of the carboxyl (C)-terminal region of KIF3A by PKA or CaMKIIα enhanced N-cadherin association with KIF3A (8). In contrast, homodimeric kinesin-2 (KIF17) promotes the release of its cargo, N-methyl-D-aspartate (NMDA) receptor 2B (GluN2B), by phosphorylation of its C-terminal region by CaMKIIα (8).

The C-terminal region of KIF3A consists of 116 amino acids. It contains a cargo-binding domain (CBD) that binds to cargo (8, 10). In this study, we screened the proteins that bind to the CBD of KIF3A by yeast two-hybrid and found Disables-1 (Dab1), which plays an important role in the intracellular adaptor protein of reelin receptors (11, 12), such as the apolipoprotein E receptor 2 (ApoER2). The interaction of KIF3A and Dab1 suggests that Dab1 may serve as a novel adaptor protein linking heterotrimeric kinesin-2 and ApoER2.

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RESULTS

Yeast two-hybrid identification of KIF3A-interacting proteins

Heterotrimeric kinesin-2 consists of motor subunits (KIF3A and KIF3B) and kinesin-associated protein 3 (KAP3), a non-motor subunit that binds KIF3 (4, 6). A previous result confirmed that the C-terminal region of KIF3A is a binding site that binds to the cargos of heterotrimeric kinesin-2 and regulates of cargo binding affinity through phosphorylation (8). To gain a better understanding of heterotrimeric kinesin-2-dependent cargo transport events, it was necessary to identify the interacting partners of the KIF3A C-terminus. Yeast two-hybrid screening was performed using KIF3A-CBD as bait (Fig. 1A). From 8×10^6 clones of the mouse brain cDNA library subcloned into the pB42AD vector, we obtained three clones containing the cDNA fragments of Dab1 (Fig. 1B).

Dab1 has a phosphotyrosine binding (PTB) domain in its amino (N)-terminal region (11, 12). All three clones isolated by yeast two-hybrid screening contained the PTB domain of Dab1 (Fig. 1B). Therefore, we investigated whether only the PTB domain of Dab1 interacts with KIF3A. As shown in Fig. 1B, a fragment of the PTB domain-containing region interacts with KIF3A. This result suggests that Dab1 interacts with KIF3A through its PTB domain containing region.

A previous study has confirmed that three sites, S689, T694, and S698, in the C-terminal tail region of KIF3A are phosphorylated (8). This phosphorylation of the KIF3A directly enhances the binding affinity of N-cadherin to heterotrimeric kinesin-2 (8). To confirm the effect of this phosphorylation of the C-terminus of KIF3A on the interaction of Dab1 with KIF3A, different fragments deleted at each of the three phosphorylation sites of KIF3A were tested for interaction with Dab1 using a yeast two-hybrid assay (Fig. 1C). The results suggest that the interaction of KIF3A with Dab1 is not affected by the three phosphorylation sites of KIF3A.

Next, we examined whether KIF3B, KIF5B (kinesin-1 motor protein), KIF17 (homodimeric kinesin-2), and KAP3 interact with Dab1. As shown in Fig. 2A, KIF3B, KIF5B, KIF17 and KAP3 did not interact with Dab1. In addition, the interaction of KIF3A with other proteins was also investigated. As shown in Fig. 2B, KIF3A interacts with Dab1. However, KIF3A did not interact with glycogen synthase kinase 3 (GSK3)-α, and the cytoplasmic region of ApoER2. Calcium-calmodulin-dependent kinase IIα (CaMKIIα) was used as a positive control (8). A quantitative β-galactosidase assay also showed that KIF3A bound to Dab1 (Fig. 2C). Taken together, these results show that Dab1 specifically interacts only with KIF3A.

Heterotrimeric kinesin-2 is associated with Dab1 in cells

The direct interaction between KIF3A and Dab1 was further confirmed by GST pull-down using purified GST-Dab1 and

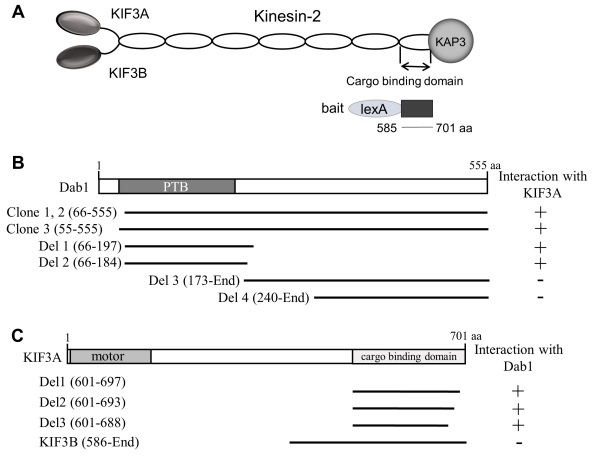

Fig. 1. Identification of the proteins that interact with KIF3A by yeast two-hybrid screening. (A) Schematic diagram of heterotrimeric kinesin-2. The CBD of KIF3A used for the yeast two-hybrid screen. (B) Specific interaction of KIF3A with the PTB domain of Dab1. Different truncations of Dab1, clone 1, clone 2, and clone 3 were tested in the yeast two-hybrid assay for interaction with KIF3A. The PTB domain-containing region of Dab1 interacted with KIF3A. (C) Dab1 binding region of KIF3A. The CBD of KIF3A is shown in gray. The truncated forms of KIF3A were tested for interaction with Dab1 in the yeast two-hybrid assay. The CBD-containing region of KIF3A specifically interacted with Dab1. +, interaction with Dab1; −, no interaction with Dab1; KIF3A, kinesin superfamily protein 3A; KAP3, kinesin superfamily associated protein 3; Dab1, Disables-1; CBD, cargo-binding domain; PTB, phosphotyrosine binding; aa, amino acids.

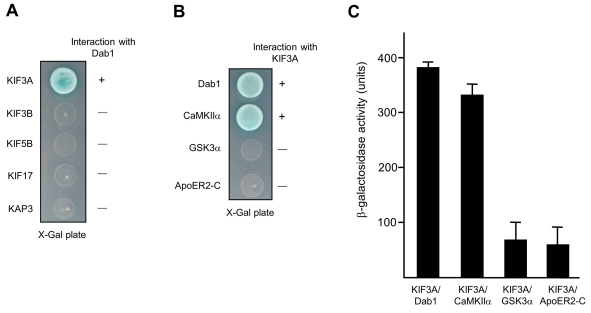

Fig. 2. Interaction of KIF3A or KIFs with Dab1. (A) The C-terminal region of each KIF was tested for interaction with full-length Dab1 in the yeast two-hybrid system. KIF3A, but not KIFs, specifically interacted with Dab1. +, interaction with Dab1; −, no interaction with Dab1. (B) Dab1, CaMKIIα, GSK3α, or ApoER2 were tested for the interaction with KIF3A in the yeast two-hybrid system. KIF3A interacted with Dab1, and CaMKIIα, but not with GSK3α, and ApoER2. +, interaction with KIF3A; −, no interaction with KIF3A. (C) The strength of the interaction of KIF3A with Dab1 was quantitatively examined using the activity of β-galactosidase in a yeast two-hybrid reporter assay. KIF3A, kinesin superfamily protein 3A; KIF3B, kinesin superfamily protein 3B; KIF5B, kinesin superfamily proteins 5B; KIF17, kinesin superfamily proteins 17; KAP3, kinesin superfamily-associated protein 3; Dab1, Disables-1; CaMKIIα, calcium-calmodulin-dependent kinase II a; GSK3α, glycogen synthase kinase-3 alpha; ApoER2, apolipoprotein E receptor 2.

His-KIF3A. As shown in Fig. 3A, KIF3A interacted with Dab1 and CaMKIIα. However, KIF3A did not interact with the cytoplasmic region of ApoER2. This result revealed a direct interaction between KIF3A and Dab1.

To further confirm the interaction of KIF3A with Dab1 in mammalian cells, co-immunoprecipitation was performed on HEK-293T cells transfected with FLAG-Dab1 or/and myc-KIF3A. The anti-FLAG antibody precipitated KIF3A and endogenous KIF3B, but not KIF5B (Fig. 3B). In addition, the anti-myc antibody precipitated Dab1 and endogenous KIF3B (Fig. 3C). These results suggest that Dab1 associates with KIF3s in cells.

A previous study has shown that Dab1 is an intracellular adaptor protein in the reelin signaling pathway and an adaptor protein for ApoER2 (11). To investigate the relationship between KIF3A, Dab1, and ApoER2, co-immunoprecipitation was performed on HEK-293T cells transfected with FLAG-Dab1 or myc-KIF3A. The cell lysates were subjected to immunoprecipitation with anti-myc antiserum. As shown in Fig. 3D, KIF3A was found to co-precipitate with ApoER2. This result suggests that Dab1 may be a link between to KIF3A and ApoER2.

Inhibition of ApoER2 transport to the cell surface

To determine whether KIF3A and Dab1 colocalize in cells, EGFP-Dab1 was transfected into HEK-293T cells. Dab1 colocalized with KIF3A in the same region of the cells (Fig. 4A). This result suggests that Dab1 is a novel binding partner of KIF3A in cells.

To investigate whether the KIF3A-Dab1 interaction is involved in the trafficking of ApoER2R to the cell surface, we introduced the EGFP-KIF3A-Δtail, which corresponds to the deletion of the CBD of KIF3A, ApoER2 was distributed on the cell surface as well as in a perinuclear compartment, but localized differently with EGFP-KIF3A-Δtail (Fig. 4B). In contrast, a KIF3A dominant-negative construct, EGFP-KIF3A-tail, corresponding to the CBD of KIF3A, was introduced into HEK-293T cells. This construct contained the Dab1 binding region but lacked the motor domain of KIF3A. The EGFP-KIF3A-tail was diffusely distributed throughout the cytoplasm in a punctate pattern (Fig. 4C). In a previous study, EGFP-KIF3A-tail showed a similar expression pattern (13). Upon overexpression of EGFP-KIF3A-tail, ApoER2 was reduced on the cell surface but diffusely distributed in the cytoplasm and predominantly concentrated around the nucleus in a punctate pattern (Fig. 4C).

The cell surface expression level of ApoER2 was further confirmed by a surface biotinylation experiments. In KIF3A dominant-negative cells, ApoER2 was significantly reduced at the cell surface (Fig. 4D, E). These results suggest that the KIF3A-Dab1 interaction plays an important role in the trafficking of ApoER2 to the cell surface.

DISCUSSION

In this study, we newly identified Dab1 as a KIF3A-binding

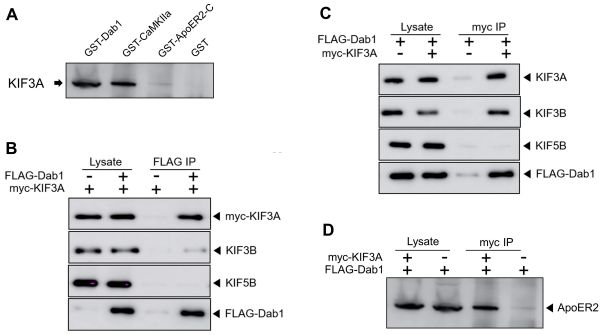

Fig. 3. Co-immunoprecipitation of KIF3A and Dab1 in cell. (A) Direct binding of Dab1 to KIF3A in a GST pull-down assay using purified GST-fused Dab1, GST-fused CaMKIIα, GST-fused ApoER2-cytoplasmic tail and His-tagged KIF3A. KIF3A directly interacts with Dab1. (B, C) HEK-293T cells were transiently transfected with myc-KIF3A and FLAG-Dab1 plasmids as indicated. Cell lysates prepared with lysis buffer containing 0.5% NP-40 were immunoprecipitated with anti-FLAG antibody in (B) or anti-myc antibody in (C). The precipitates were immunoblotted with anti-KIF3A, KIF3B, KIF5B or FLAG antibodies. Dab1 co-precipitated KIF3A and KIF3B, but not KIF5B. (D) Cell lysates were immunoprecipitated with anti-myc antibody and the precipitates were immunoblotted with anti-ApoER2 antibody. KIF3A, kinesin superfamily protein 3A; KIF3B, kinesin superfamily protein 3B; KIF5B, kinesin superfamily proteins 5B; Dab1, Disables-1; ApoER2, apolipoprotein E receptor 2.

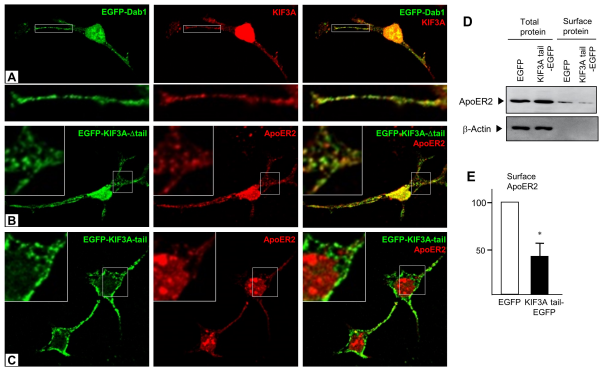

Fig. 4. KIF3A is involved in the trafficking of ApoER2 to the cell surface. (A) Twenty-four hours after transfection with EGFP-Dab1 plasmids, HEK-293T cells were subjected to immunofluorescence staining with anti-KIF3A antibody. (B, C) HEK-293T cells were transfected with EGFP-KIF3A-Δtail or EGFP-KIF3A-tail. At 72 hr after transfection, cells were fixed and stained with antibody against ApoER2. (D, E) Overexpression of KIF3A-CBD-EGFP bound to Dab1 resulted in decreased cell surface ApoER2 expression in cells. (D) Western blot analysis of cell surface ApoER2. Statistics of cell surface expression of ApoER2 in (E) (normalized to total amount, n = 3 independent experiments, *P < 0.05; Student's t test). Error bars represent mean ± SEM. KIF3A, kinesin superfamily protein 3A; KIF3B, kinesin superfamily protein 3B; KIF5B, kinesin superfamily proteins 5B; Dab1, Disables-1; ApoER2, apolipoprotein E receptor 2.

protein by yeast two-hybrid screening using the CBD of KIF3A as bait. The region containing the PTB domain of Dab1 was the minimal binding domain for KIF3A binding in the yeast two-hybrid assay. Furthermore, we showed by co-immunoprecipitation that heterotrimeric kinesin-2 can co-precipitate with Dab1 and ApoER2. When EGFP-Dab1 and myc-KIF3A were co-expressed in HEK-293T cells, Dab1 co-localized with KIF3A. We also showed that ApoER2 expression at the cell surface was reduced in KIF3A dominant-negative cells. These results suggest that the KIF3A-Dab1 interaction plays an important role in the cell surface localization of ApoER2.

Heterotrimeric kinesin-2 transports various vesicles within cells (2, 5, 7). In our previous study, microinjection and immunoprecipitation of anti-KIF3s antibodies into cells suggested that heterotrimeric kinesin-2 plays a role in the anterograde transport of membrane-bound organelles, large vesicles, and small vesicles to the plasma membrane (7). In this study, EGFP-KIF3A-tail was diffusely distributed throughout the cytoplasm in a punctate pattern (Fig. 4C). These punctates in the cytoplasm may be various vesicles that are transported by kinesin-2.

KIF3A and KIF3B share a highly conserved motor domain and coiled-coil domain (6). However, the C-terminal region of KIF3A, which contains the CBD, has low similarity to KIF3B and KIF3C (6, 8). In this study, Dab1 was interacted with the CBD of KIF3A. A previous study reported that phosphorylation of heterotrimeric kinesin-2 regulates cargo interaction (8). Phosphorylation of KIF3A stimulates cargo interaction with heterotrimeric kinesin-2, whereas phosphorylation of homodimeric kinesin-2 disrupts cargo interaction with homodimeric kinesin-2 (8). In this study, the phosphorylation of heterotrimeric kinesin-2 did not affect Dab1 binding.

Dab1 plays a role as an adaptor protein that function in response to the extracellular protein reelin (11). Reelin binds to low-density lipoprotein receptors such as ApoER2 and the very low-density lipoprotein receptor (VLDLR) (11). When reelin binds to reelin receptors, Dab1 is phosphorylated at multiple sites by tyrosine kinases, and Dab1 binds to the C-terminal tail of reelin receptors (14). In addition, Dab1 has a scaffolding function in the Notch pathway through Dab1-mediated inhibition of Notch cytoplasmic domain degradation (15). In a previous study, Dab1 interacted with either amyloid precursor protein (APP) or ApoER2 to increase the cell surface levels and decrease amyloid beta (Aβ) levels (16). A genetic polymorphism study suggests that mutations in the ApoER2 gene may be associated with the development of Alzheimer's disease (17). The association between ApoER2 and Alzheimer's disease has been reported as dysfunction of ApoER2 trafficking in cells as a risk factor for familial Alzheimer's disease (18). In this study, ApoER2 cell surface expression was significantly reduced in KIF3A dominant negative cells (Fig. 4D, E). This result suggests that kinesin-2 may be involved in ApoER2-related diseases.

What is the significance of the interaction between KIF3A and Dab1? One possibility is that Dab1 may act as an adaptor protein that links heterotrimeric kinesin-2 to ApoER2. In many cases, kinesins interact with different cargoes using the many different adaptor proteins (3, 4). More than 20 adaptor proteins have been identified that bind to the kinesins (4, 19). The adaptor proteins that interact with kinesin-1 are involved in the transport of a variety of cargoes (3, 4). The c-Jun NH_2-terminal kinase (JNK)-interacting protein 1 (JIP1) was the first adaptor protein identified for kinesin-1 (20). JIP1 serves as an adaptor protein that links the kinesin light chain (KLC) of the kinesin-1 to cargoes such as APP (4, 19). Previous study has shown that kinesin-1 binds to γ-aminobutyric acid (GABA) type A receptors via GABA type A receptor-related protein proteins (21). The many different adaptor proteins for KIFs are thought to reflect cargo interaction specificity and delivery to specific targets within cells (3, 4, 22). In previous findings, Dab1 was identified as an intracellular adaptor protein in the reelin signaling pathway and plays a key role in the intracellular trafficking of reelin receptors, including ApoER2 (11, 14). Our results suggest that Dab1 may serve as an adaptor protein linking heterotrimeric kinesin-2 to ApoER2, and that the KIF3A-Dab1 interaction may play an important role in the localization of ApoER2 in cells.

Another possibility is that KIF3A-Dab1 interaction is an adaptor protein that plays a role in the signaling pathway that is important for the proper development and function of the brain. In previous report, heterotrimeric kinesin-2 plays a role in involvement in signaling pathway and developmental processes (2). For example, heterotrimeric kinesin-2 interacts with components of the Wnt signaling pathway, which is involved in embryonic development (2, 5). Other studies have linked heterotrimeric kinesin-2 to the Sonic hedgehog (Shh) signaling pathway, which is critical for embryonic development (5). Our findings provide insight into the possible transport of ApoER2-containing cargo or downstream components of the reelin signaling pathway by kinesin-2 through Dab1 and KIF3A interaction. Further studies are needed to fully understand the interaction between the heterotrimeric kinesin-2 and Dab1 and the intracellular trafficking of ApoER2.

MATERIALS AND METHODS

Plasmid constructs

The KIF3A cDNA (gift from K. Kaibuchi, Nagoya University, Graduate School of Medicine, Japan) (23) fragment corresponding to the CBD (amino acids 585-701) was amplified using appropriate primers. The amplified fragment was cloned into pLexA (Clontech Laboratories, Inc., Palo Alto, CA, USA). The resulting recombinant plasmid, pLexA-CBD-KIF3A, was used as a bait plasmid for yeast two-hybrid screening. The C-terminal region of KIF3A, and Dab1 (gift of S. Hisanaga, Tokyo Metropolitan University, Japan) (11) were amplified by PCR and cloned into pLexA and pB42AD (clontech).

Screening of KIF3A-binding proteins by yeast two-hybrid assay

The Matchmaker LexA two-hybrid system was used for screen-

ing according to the manufacturer's instructions (Clontech). Briefly, pLexA-CBD of KIF3A was transformed into yeast strain EGY48 carrying the p8op-lacZ gene. Transformed EGY48 yeast cells containing pLexA-CBD of KIF3A were transformed with a mouse brain cDNA library (21). Unique inserts were sequenced and the DNA sequence analysed using the BLAST algorithm at the National Center for Biotechnology Information (NCBI).

β-Galactosidase activity in liquid cultures of yeast

Yeast β-galactosidase activity was assayed as previously described (24). Briefly, mid-log phase yeast cells were harvested and permeabilized with 0.1% sodium dodecyl sulfate (SDS) and chloroform. An excess of o-nitrophenyl-β-D-galactoside (ONPG) (Sigma-Aldrich, St. Louis, MO, USA) was added to the yeast lysate and the mixture was incubated at 30°C. The o-nitrophenol contained in the reaction product was determined by measuring the absorbance at 420 nm in a spectrophotometer and normalizing for reaction time and cell density. Enzyme activity units were calculated using the following equation: units = 1,000 × ((OD_{420}– 1.75 × OD_{550})) / (reaction time (min) × culture volume (ml) × OD_{600}).

In vitro glutathione S-transferase (GST) pull-down assays

To express the full-length of Dab1, the full-length of CaMKIIα, the C-terminal region of ApoER2, and His-KIF3A were cloned into pET41 or pET28, respectively, and then into bacterial strain BL21 GOLD (Stratagene, La Jolla CA, USA). Recombinant protein-bound glutathione-Sepharose 4B (GE Healthcare, Saint Louis, MO, USA) was washed three times with washing buffer (50 mM Tris-HCl, pH 8.0, and 100 mM NaCl) containing 1 mM PMSF and 1 mM DTT. His-KIF3A was applied to bead-immobilized GST or GST-Dab1, GST-CaMKIIα, and GST-ApoER2-C. After incubation for 1 hr at room temperature, the beads were washed with washing buffer. Bound proteins analyzed by Western blot using anti-KIF3A antibody (Abcam, Cambridge, MA, USA).

Cell culture and transfection

HEK-293T cells (American Type Culture Collection [ATCC] CRL-3216) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, L-glutamine, and antibiotics. Transient transfection was performed using the $CaPO_4$ precipitation method as previously described (24).

Co-immunoprecipitation and immunoblot analysis

Twenty-four hours after transfection with the myc-KIF3A and FLAG-Dab1 constructs, HEK-293T cells were rinsed twice with ice-cold PBS and then lysed with ice-cold lysis buffer (PBS containing 0.5% NP-40 and 1x protease inhibitor cocktail set V [Calbiochem, San Diego, CA, USA]) by gentle rotation for 30 min. The cell lysate was incubated with anti-FLAG M2 agarose beads (Sigma) or anti-myc agarose beads (Sigma) for 2 hr at 4°C with constant shaking. The beads were collected by centrifugation at 2,000 × *g* for 30 sec and washed 5 times with ice-cold PBS containing 0.5% NP-40. Each sample was processed for SDS-PAGE and immunoblot analysis using antibodies against KIF3A, KIF3B (Sigma), KIF5B (Abcam), ApoER2 (Abcam), and FLAG (Sigma).

Immunocytochemistry

HEK-293T cells grown on poly-D-lysine-coated coverslips were transfected with the EGFP-Dab1 construct. Twenty-four hours after transfection, the cells were washed with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde, and permeabilized with 0.2% Triton X-100 in PBS for 10 min. Cells were incubated with anti-KIF3A diluted 1:500 in PBS containing 1% bovine serum albumin (BSA) and 0.05% Tween-20 at 4°C for overnight. After washing with PBS, the cells were incubated with Dylight 594-conjugated goat anti-rabbit IgG antibody (Jackson ImmunoResearch Labs, West Grove, PA, USA) diluted 1:800 for 40 min. After washing with PBS, cells were mounted with Fluoromount (DAKOKorea, Seoul, Korea). Fluorescence images were captured with a Zeiss LSM510 META confocal laser scanning microscope (Carl Zeiss Inc, Oberkochem, Germany).

Cell surface protein analysis

For cell surface protein analysis, cell surface proteins from HEK-293T cells cultured for 3-4 days in 6-cm diameter dishes were biotinylated and extracted using a cell surface protein isolation kit (Pierce, Rockford, IL, USA). The extracted proteins were then analyzed by immunoblotting. Quantification of band intensities was performed using Image J software.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

REFERENCES

1. Hirokawa N (1988) Kinesin and dynein superfamily proteins and the mechanism of organelle transport. Science 279, 519-526
2. Hirokawa N (2000) Stirring up development with the heterotrimeric kinesin KIF3. Traffic 1, 29-34
3. Seog DH, Lee DH and Lee SK (2004) Molecular motor proteins of the kinesin superfamily proteins (KIFs): structure, cargo and disease. J Korean Med Sci 19, 1-7

4. 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
5. Scholey JM (2013) Kinesin-2: a family of heterotrimeric and homodimeric motors with diverse intracellular transport functions. Annu Rev Cell Dev Biol 29, 443-469
6. Yamazaki H, Nakata T, Okada Y and Hirokawa N (1996) Cloning and characterization of KAP3: a novel kinesin superfamily-associated protein of KIF3A/3B. Proc Natl Acad Sci U S A 93, 8443-8448
7. Takeda S, Yamazaki H, Seog DH et al (2000) Kinesin superfamily protein 3 (KIF3) motor transports fodrin-associating vesicles important for neurite building. J Cell Biol 148, 1255-1265
8. Ichinose S, Ogawa T and Hirokawa N (2015) Mechanism of activity-dependent cargo loading via the phosphorylation of KIF3A by PKA and CaMKIIα. Neuron 87, 1022-1035
9. Kovacs JJ, Whalen EJ, Liu R et al (2008) Beta-arrestin-mediated localization of smoothened to the primary cilium. Science 320, 1777-1781
10. Kodani A, Salomé Sirerol-Piquer M, Seol A et al (2013) Kif3a interacts with Dynactin subunit p150 Glued to organize centriole subdistal appendages. EMBO J 32, 597-607
11. Fuchigami T, Sato Y, Tomita Y et al (2013) Dab1-mediated colocalization of multi-adaptor protein CIN85 with Reelin receptors, ApoER2 and VLDLR, in neurons. Genes Cells 18, 410-424
12. Margolis B, Borg JP, Straight S and Meyer D (1999) The function of PTB domain proteins. Kidney Int 56, 1230-1237
13. Yoshihara S, Jiang X, Morikawa M et al (2021) Betaine ameliorates schizophrenic traits by functionally compensating for KIF3-based CRMP2 transport. Cell Rep 35, 108971
14. Lane-Donovan C and Herz J (2017) ApoE, ApoE receptors, and the synapse in Alzheimer's disease. Trends Endocrinol Metab 28, 273-284
15. Honda T and Nakajima K (2016) Proper level of cytosolic Disabled-1, which is regulated by dual nuclear translocation pathways, is important for cortical neuronal migration. Cereb Cortex 26, 3219-3236
16. Hoe HS, Tran TS, Matsuoka Y, Howell BW and Rebeck GW (2006) DAB1 and Reelin effects on amyloid precursor protein and ApoE receptor 2 trafficking and processing. J Biol Chem 281, 35176-35185
17. Ma SL, Ng HK, Baum L et al (2002) Low-density lipoprotein receptor-related protein 8 (apolipoprotein E receptor 2) gene polymorphisms in Alzheimer's disease. Neurosci Lett 332, 216-218
18. Wang W, Moerman-Herzog AM, Slaton A and Barger SW (2017) Presenilin 1 mutations influence processing and trafficking of the ApoE receptor ApoER2. Neurobiol Aging 49, 145-153
19. Sun F, Zhu C, Dixit R and Cavalli V (2011) Sunday Driver/JIP3 binds kinesin heavy chain directly and enhances its motility. EMBO J 30, 3416-3429
20. Verhey KJ, Meyer D, Deehan R et al (2001) Cargo of kinesin identified as JIP scaffolding proteins and associated signaling molecules. J Cell Biol 152, 959-970
21. Nakajima K, Yin X, Takei Y et al (2012) Molecular motor KIF5A is essential for GABA(A) receptor transport, and KIF5A deletion causes epilepsy. Neuron 76, 945-961
22. Nabb AT, Frank M and Bentley M (2020) Smart motors and cargo steering drive kinesin-mediated selective transport. Mol Cell Neurosci 103, 103464
23. Nishimura T, Kato K, Yamaguchi T et al (2004) Role of the PAR-3-KIF3 complex in the establishment of neuronal polarity. Nat Cell Biol 6, 328-334
24. Jang WH, Jeong YJ, Choi SH et al (2014) Dynamin-1-like protein (Dnm1L) interaction with kinesin light chain 1 (KLC1) through the tetratricopeptide repeat (TPR) domains. Biosci Biotechnol Biochem 78, 2069-2072