ISSN (Print) 1225-9918
ISSN (Online) 2287-3406
Journal of Life Science 2016 Vol. 26. No. 8. 963~969 DOI : http://dx.doi.org/10.5352/JLS.2016.26.8.963

The Scaffolding Protein WAVE1 Associates with Kinesin 1 through the Tetratricopeptide Repeat (TPR) Domain of the Kinesin Light Chain (KLC)

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Received April 14, 2016 / Revised May 17, 2016 / Accepted June 22, 2016

Kinesin superfamily proteins (KIFs) are microtubule-dependent molecular motor proteins essential for the intracellular transport of organelles and protein complexes in cells. Kinesin 1 is a member of those KIFs that transport various cargoes, including organelles, synaptic vesicles, neurotransmitter receptors, cell signaling molecules, and mRNAs through interaction between its light chain subunit and the cargoes. Kinesin light chains (KLCs) are non-motor subunits that associate with the kinesin heavy chain (KHC) dimer. KLCs interact with many different binding proteins, but their particular binding proteins have not yet been fully identified. We used the yeast two-hybrid assay to identify proteins that interact with the tetratricopeptide repeat (TPR) domain of KLC1. We found an interaction between the TPR domain of KLC1 and Wiskott-Aldrich syndrome protein family member 1 (WAVE1), a member of the WASP/WAVE family involved in regulation of actin cytoskeleton. WAVE1 bound to the six TPR domain-containing regions of KLC1 and did not interact with KHCs (KIF5A, KIF5B, and KIF5C) in the yeast two-hybrid assay. The carboxyl (C)-terminal verprolin-cofilin-acidic (VCA) domain of WAVE1 is essential for interaction with KLC1. Also, other WAVE isoforms (WAVE2 and WAVE3) interacted with KLC1 in the yeast two-hybrid assay. When co-expressed in HEK-293T cells, WAVE1 co-localized with KLC1 and co-immunoprecipitated with KLC1 and KIF5B. These results suggest that kinesin 1 motor protein may transport WAVE complexes or WAVE-coated cargoes in cells.

Key words : Kinesin 1, KLC1, Microtubule motor, TPR domain, WAVE

Introduction

The intracellular transport is mediated by microtubule motor proteins. These motor proteins act in several processes in the brain, including neuronal function, development, and plasticity, by regulating the intracellular transport within the axons and dendrites [5, 15]. Kinesin superfamily proteins (KIFs) are microtubule-dependent motor proteins involved in the transport of various cargoes, including membrane vesicles, organelles, proteins complexes, and mRNAs [6]. Kinesin 1 is the first identified member of KIFs responsible for an ATP-dependent, anterograde fast axonal transport [7]. Kinesin 1 is a heterotetrameric protein of two heavy chains (KHCs, also called KIF5s) and two light chains (KLCs) [5, 7]. KHCs contain three domains: an amino (N)-terminal motor domain, a central coiled-coil domain, and a carboxyl (C)-terminal domain that regulates motor activity [5]. KLCs are non-motor proteins that associate with KHCs. KLCs consist of the N-terminal coiled-coil domain that binds to KHC, the central tetratricopeptide repeat (TPR) domains, and the C-terminal domain [4]. The TPR domains of KLCs are highly conserved across species and are known to be involved in protein-protein interactions [23]. The TPR domains have been suggested to be involved in cargo interaction [4, 8].

The TPR domain is well known as a module playing a role as an organizing center for complexes regulating a number of biological processes [23]. It consists of 34 amino acids which assemble into a characteristic helix-turn-helix structure for each TPR repeat [2]. The first protein identified to bind to the TPR domain was the c-Jun NH_2-terminal kinase (JNK)-interacting protein (JIP, also known as JSAP) group of scaffold proteins [14, 15]. The three JIP isoforms in mammals are scaffolds for the MAP kinase cascade that activates JNK [4]. Many different types of cargoes moved by kinesin 1 have been identified, including mitochondria, neurotransmitter receptor containing vesicle, virus particle, and mRNA granules [7, 8, 11]. In some cases, these cargoes bind

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to soluble adaptor proteins/scaffolding proteins that mediate the attachment to kinesin 1 [6, 8].

Understanding how kinesin 1 becomes linked to particular cargo and how protein complex assemble on scaffolds at their final destination remain to be unsolved question. In this study, we screened for proteins that interact specifically with the six TPR domains of KLC1 and found the interaction with the Wiskott-Aldrich syndrome protein family member 1 (WAVE1), the scaffolding protein that associates with numerous partner proteins, including Arp2/3 complex, phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2), G-actin, and WAVE-associated Rac GTPase-activating protein (WRP) [20]. The KLC1 and WAVE1 interaction suggests that WAVE1 complex may be transported by kinesin 1, or play a role as a scaffold for between kinesin 1 and WAVE-bound cargoes.

Materials and Methods

Plasmid constructs

The mouse KLC1 cDNA fragment corresponding to the six TPR domains (amino acids 80-541) was amplified using the appropriate primers [9, 10]. The amplified fragment was cloned into pLexA (Clontech, Palo Alto, CA, USA). The resulting recombinant plasmid, pLexA-6xTPR-KLC1, was used as bait plasmid. The full-length cDNAs of mouse WAVE1 (GeneBank accession number: NM_031877), WAVE2 (GeneBank accession number: NM_153423), and WAVE3 (GeneBank accession number: NM_145155) were amplified by polymerase chain reaction (PCR) from Marathon-Ready™ cDNA library (Clontech) and cloned into pB42AD (Clontech).

Screening of KLC1-binding proteins by yeast two-hybrid assay

The Matchmaker LexA two-hybrid system was used for screening according to the manufacturer's manual (Clontech). In brief, pLexA-6xTPR-KLC1 was transformed into yeast strain EGY48 carrying the p8op-lacZ gene. The transformed EGY48 yeast cells containing pLexA-6xTPR-KLC1 were transformed with the mouse brain cDNA library [9] 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 restriction digestion. 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 the retransformation.

β-Galactosidase activity in liquid cultures of yeast

The β-galactosidase activity of yeast was assayed as described previously [10]. In brief, mid-log phase yeast cells were collected and permeabilized with 0.1% sodium dodecyl sulphate (SDS) and chloroform. An excess amount of o-nitrophenyl-β-D-galactoside (ONPG) was added to yeast lysate, the mixture was incubated at 30℃, 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 and the cell density. The units of enzyme activity were calculated by the following equation: units = 1,000 × [(OD_{420} − 1.75 × OD_{550})] / [reaction time (min) × culture volume (ml) × OD_{600}]. All experiments were independently performed at least three times [10].

Cell culture and transfection

HEK-293T cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, l-glutamine, and antibiotics. Transient transfections were done with the $CaPO_4$ precipitation method.

Immunocytochemistry

HEK-293T cells grown on poly-D-lysine-coated coverslips were transfected with EGFP-WAVE1 and KLC1 constructs. Twenty-four hours after transfection, cells were washed with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde in PBS for 5 min, and permeabilized with 0.2% Triton X-100 in PBS for 10 min. After blocking with 5% normal goat serum in PBS for 30 min, cells were incubated overnight at 4°C with anti-KLC antibody (Millipore, Billerica, MA, USA) diluted 1:500 in PBS containing 1% bovine serum albumin (BSA) and 0.05% Tween-20. After washing 3 times with PBS, cells were incubated for 40 min with Dylight 594-conjugated goat anti-rabbit IgG antibody (Jackson ImmunoResearch Labs, West Grove, PA, USA) diluted 1:800. After washing 3 times with PBS, the cells were mounted with Fluoromount (DAKO). Fluorescence images were acquired on Zeiss LSM510 META confocal laser scanning mi-

croscope (Carl Zeiss, Oberkochem, Germany).

Co-immunoprecipitation and immunoblot analysis

Twenty-four hours after transfection with myc-KLC1 and FLAG-WAVE1 constructs, HEK-293T cells were rinsed with ice-cold PBS twice and lysed with ice-cold lysis buffer [PBS containing 0.5% NP-40 and 1x protease inhibitor cocktail set V (Calbiochem)] by gentle rotation for 30 min. Lysates were centrifuged at 16,000 × *g* for 10 min at 4°C. The supernatant was incubated with anti-FLAG M2 agarose beads (Sigma-Aldrich) for 2 h 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. The washed beads were resuspended with Laemmli's loading buffer and the proteins were eluted and denatured by boiling for 2 minutes and then processed for SDS-PAGE and immunoblot analysis with antibodies against KLC1, KIF5B, and KIF3A [9].

Results

Identification of KLC1-interacting proteins by yeast two-hybrid screening

To identify the binding proteins of KLC1, we screened a mouse brain cDNA library through the yeast two-hybrid assays using the six TPR domains-containing region (amino acids 80-541) as bait (Fig. 1B). From 8 × 10^6 colonies screened, we obtained 2 positive clones. Both the positive clones turned out to possess WAVE1 cDNA fragments (Fig. 1A). The clones overlapped at the open reading frame (ORF) of WAVE1 and possessed cDNA fragments corresponding to the C-terminal verprolin/cofilin/acidic (VCA) domain of WAVE1 (Fig. 1A). KLC1 is composed of an N-terminal domain, a C-terminal domain, and the central six TPR domains, protein-binding modules present in several proteins (Fig. 1B) [2, 4, 5]. To determine the minimal binding domain of KLC1 that is required for the interaction with WAVE1, we constructed several deletion mutants of KLC1. Yeast two-hybrid assays showed that the binding with WAVE1 was dependent on the region containing the six TPR domains of KLC1 (Fig.

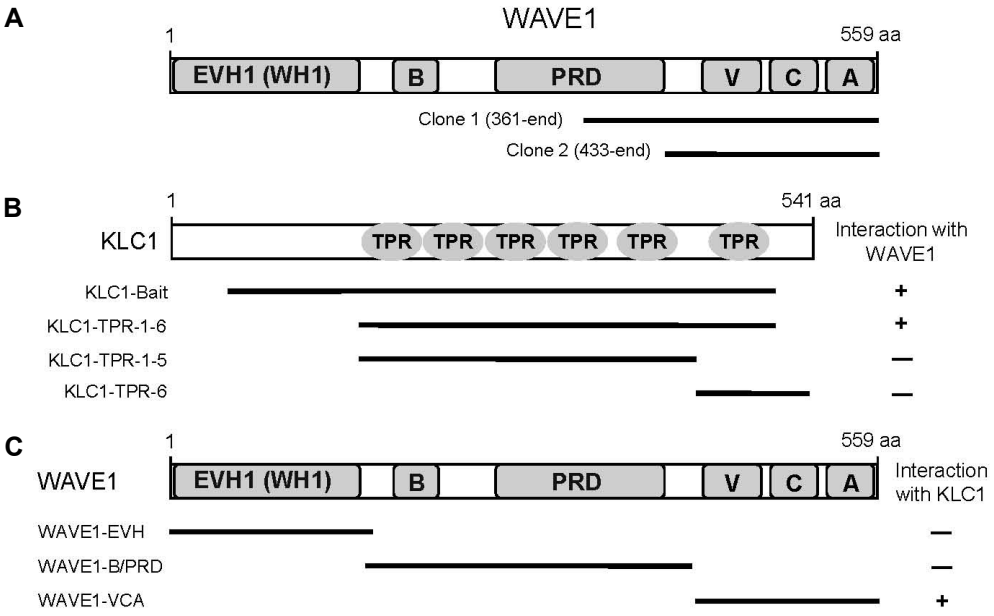

Fig. 1. Identification of the proteins interacting with KLC1 by yeast two-hybrid screening. (A) Schematic diagram of WAVE1. The gray boxes indicate the Ena-VASP-homology-1 (EVH1; also known as WH1) domain, the basic (B) domain, the proline-rich domain (PRD), the verprolin/cofilin/acidic (VCA) domain of WAVE1. Clones 1 and 2 were isolated from the yeast two-hybrid screen and were overlapped at the C-terminal region of WAVE1. aa, the amino acid residue number. (B) Minimal WAVE1 binding region in KLC1. KLC1 has six TPR domains, indicated in gray. Several truncated forms of KLC1 were constructed by PCR and were tested in the yeast two-hybrid assay for interaction with WAVE1. aa, the amino acid residue number. +, interaction with WAVE1; -, no interaction with WAVE1. (C) Minimal KLC1 binding region in WAVE1. Several truncated forms of WAVE1 were constructed by PCR and tested in the yeast two-hybrid assay for interaction with KLC1. aa, the amino acid residue number. +, interaction with KLC1; -, no interaction with KLC1.

1B). WAVE1 was originally discovered as a mutated gene in Wiskott-Aldrich syndrome, a recessive X-linked immunodeficiency disorder [18]. It is a multi-domain protein comprised of a Wiskott-Aldrich syndrome protein (WASP) homology (WH1, also called EVH1) domain, a basic region (B), a GTPase-binding domain (GBD), a proline-rich domain (PRD), and VCA domain [18, 20]. To identify the domain of WAVE1 required for the interaction with KLC1, a series of deletion mutants of WAVE1 was constructed and analyzed their interactions with KLC1 using the yeast two-hybrid assay. Only the C-terminal VCA domain of WAVE1 interacted with KLC1 in the yeast two-hybrid assay (Fig. 1C). This experiment demonstrated that the minimal binding domain was located in the C-terminal VCA domain of WAVE1.

To clarify whether WAVE1 interacts with only KLC1 or with other kinesin 1 subunits, the C-terminal tails of KIF5A, KIF5B, and KIF5C were tested for binding with WAVE1. As shown in Fig. 2A, WAVE1 did not interact with the C-terminal tails of KIF5A, KIF5B, and KIF5C. Next, we investigated whether KLC1 interacts with the other two WAVEs, WAVE2 and WAVE3. As shown in Fig. 2B, KLC1 also interacted with WAVE2 and WAVE3 in yeast two-hybrid assay. The result is not surprising because the C-terminal VCA domains of WAVE1, WAVE2, and WAVE3 share extensive similarity in their amino acid sequences (80% identity between WAVE1 and WAVE2, 83% identity between WAVE1 and WAVE3) [18]. To quantify the binding affinity of KLC1 to WAVE1, the WAVE1 plasmid and the KLC1 or KIF5s expression plasmids were transformed to yeast and the β-galactosidase activity was measured in liquid cultures. The interaction of KLC1 with WAVE1 yielded approximately 392 units of β-galactosidase activity (Fig. 2C).

WAVE1 is associated with kinesin 1 in cells

The KLC1-WAVE1 interaction was also assessed in mammalian cells. When the lysate of HEK-293T cells transiently expressing myc-KLC1 and FLAG-WAVE1 was immunoprecipitated with anti-FLAG antibody, KLC1 and KIF5B, but not KIF3A, a motor subunit of kinesin 2 were co-precipitated with WAVE1 (Fig. 3A). These results indicate that WAVE1 associates with kinesin 1. In order to further confirm whether KLC1 and WAVE1 co-localize in cells, KLC1 was co-expressed with EGFP-WAVE1 in HEK-293T cells. KLC1 and WAVE1 co-localized at the same region in cells (Fig. 3B). Taken together, these results indicate that WAVE1 interacts with kinesin 1 through the binding with KLC1 subunit.

Discussion

In this study, we show that KLC1 interacts with WAVE1, as a binding protein. Using the TPR domains-containing region of KLC1 as bait, we identified WAVE1 in a yeast two-hybrid assay of a mouse brain cDNA library. The C-terminal VCA domain of WAVE1 interacts with the six TPR domains-containing region of KLC1. Furthermore, when KLC1 and WAVE1 were expressed in mammalian cells, they

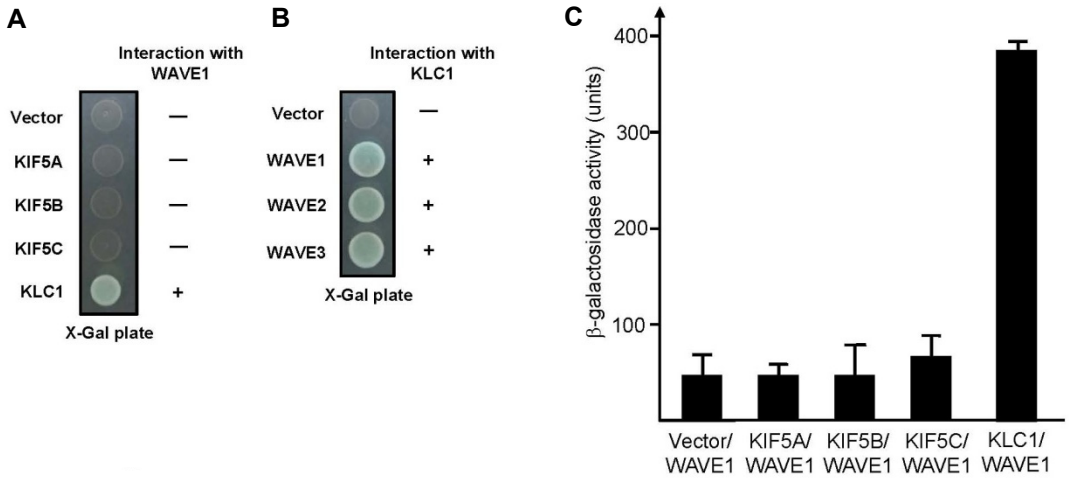

Fig. 2. Interaction between KLC1 and WAVEs. The tail region of each KIF5 and the full length KLC1 were fused to the pLexA DNA binding domain. (A) WAVE1 specifically interacted with KLC1 but not with KIF5s. +, interaction with WAVE1; -, no interaction with WAVE1. (B) KLC1 interacted with all three WAVEs. +, interaction with KLC1; -, no interaction with KLC1. (C) The strength of interactions between WAVE1 and KIF5s or KLC1 was examined quantitatively using β-galactosidase activity in yeast two-hybrid reporter assay.

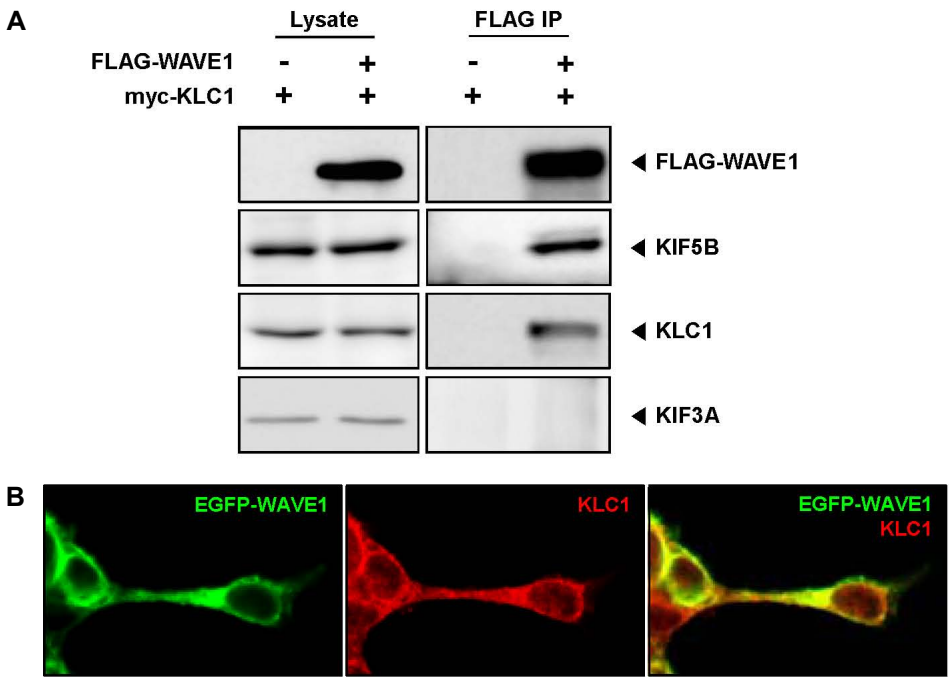

Fig. 3. Co-immunoprecipitation and co-localization of KLC1 and WAVE1 in mammalian cells. (A) HEK-293T cells were transiently transfected with FLAG-WAVE1 and either control vector or myc-KLC1 as indicated. Cell lysates were incubated with monoclonal anti-FLAG antibody to immunoprecipitate WAVE1. Immunoblots were subsequently probed for KLC1, KIF5B, KIF3A, and WAVE1. WAVE1 specifically co-immunoprecipitated with kinesin 1. (B) HEK-293T cells were transiently transfected with EGFP-WAVE1 and KLC1 plasmids. Twenty-four hours after transfection, cells were immunostained using anti-KLC1 antibody. KLC1 and EGFP-WAVE1 are seen at the same subcellular region of cells.

co-immunoprecipitated and co-localized in cells. Taking all of these results together, we hereby propose that KLC1 and WAVE1 interaction may have role in formation of an adaptor protein complex for transport by kinesin 1.

Several kinesin 1 cargo molecules have been shown to interact with the TPR domain of KLCs [4]. The TPR domains are known as a protein-protein interaction module, functioning as a physical linker between the KIF5s and various cargoes. The TPR domains or other region of KLC1 interact with over 10 different cargoes, including the c-Jun N-terminal kinase-interacting protein 1 (JIP1), dynamin-1-like protein (Dnm1L), nesprins, neuronal cell adhesion molecules (NCAMs), adenovirus E1B 19k protein-interacting protein 2 (BNIP-2), and protein interacting with APP tail 1 (PAT1) [1, 8, 10, 14, 21, 22]. In this study, we have identified that WAVE1 interacted with the TPR domain of KLC1.

WAVE1 was first identified as a mutated gene in the Wiskott-Aldrich syndrome, a recessive X-linked immunodeficiency disorder [18]. It plays a role as a module that permits the assembly of multi-protein complexes [20]. Previous proteomic and biochemical studies have shown that the EVH1 domain of WAVE1 binds a core unit of four proteins that regulate Rac signaling at sites of actin reorganization [13, 16]. The PRD domain of WAVE1 interacts with Src homology 3 (SH3) domain-containing proteins including Abelson tyrosine kinase (Abl) [20]. The principal function of the VCA domain of WAVE1 is to bind the Arp2/3 complex and initiate the rapid polymerization of actin filaments, which is necessary for cell motility and migration [13, 20]. In this study, we have shown that KLC1 also interacted with the VCA domain of WAVE1.

What would the interaction between KLC1 and WAVE1 mean? One possibility is that WAVE1 may be an adaptor protein that links kinesin 1 and membranous cargoes. The B domain of WAVE1 binds to PIP_2 [12]. This binding is important for the membrane localization of WAVE1. WHAMM, the novel member of WAVE family localizes to the tubular structures out of the endoplasmic reticulum and assists with the movement of vesicles during anterograde transport [3]. Although we did not determine the specific character of WAVE1-coated kinesin 1 cargo, the available data suggest that WAVE1 play a role as an adaptor protein for membranous cargo transport by kinesin 1. Another possibility is that kinesin 1 transport the WAVE1-associated signaling

protein complex that links upstream signals to the activation of Arp2/3 complex involved in actin polymerization. WAVEs form a pentameric heterocomplex (referred to as the WAVE complex) with Abelson-interacting protein (ABI), non-catalytic tyrosine kinase (Nck)-associated protein 1 (NAP1), specifically Rac-associated protein 1 (SRA1), and haematopoietic stem/progenitor cell protein 300 (HSPC300) [20]. Extracellular stimuli resulting in the dissolution of WAVE complex lead to Arp2/3-mediated actin polymerization [17, 20]. Rapid actin polymerization is induced at the leading edge of the membrane protrusion, resulting in formation of filopodia and lamellipodia by CDC42 and Rac, respectively [16, 20]. Interestingly, WAVE2 importantly involved in the regulation of lamellipodia formation was transported to the leading edge by KIF5B along microtubules [19]. WAVE1 also binds WRP, which plays a role in the control the membrane protrusion [16]. A previous study had shown that a reduction in the amount of WRP interacting with WAVE1 affected the dendritic morphology of neurons and their synaptic transmission in the mutant mice [16]. Our findings provide new insight into the mechanism of kinesin 1-mediated transport, by the direct interaction between KLC1 and WAVE1, of WAVE complex playing a central role in membrane and actin dynamics.

Acknowledgment

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 (NRF-2015R1D1A1A01056820).

References

1. Akamatsu, R., Ishida-Kitagawa, N., Aoyama, T., Oka, C. and Kawaichi, M. 2015. BNIP-2 binds phosphatidylserine, localizes to vesicles, and is transported by kinesin-1. *Genes Cells* **20,** 135-152.
2. Blatch, G. L. and Lässle, M. 1999. The tetratricopeptide repeat: a structural motif mediating protein-protein interactions. *Bioessays* **21**, 932-939.
3. Campellone, K. G., Webb, N. J., Znameroski, E. A. and Welch, M. D. 2008. WHAMM is an Arp2/3 complex activator that binds microtubules and functions in ER to Golgi transport. *Cell* **134**, 148-161.
4. Gindhart, J. G. 2006. Towards an understanding of kinesin-1 dependent transport pathways through the study of protein-protein interactions. *Brief Funct. Genomic. Proteomic.* **5**, 74-86.
5. Hirokawa, N. 1998. Kinesin and dynein superfamily proteins and the mechanism of organelle transport. *Science* **279**, 519-526.
6. Hirokawa, N. and Noda, Y. 2008. Intracellular transport and kinesin superfamily proteins, KIFs: structure, function, and dynamics. *Physiol. Rev.* **88**, 1089-1118.
7. Hirokawa, N. and Takemura, R. 2005. Molecular motors and mechanisms of directional transport in neurons. *Nat. Rev. Neurosci.* **6**, 201-214.
8. 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.
9. Jang, W. H., Jeong, Y. J., Choi, S. H., Kim, S. J., Urm, S. H. and Seog, D. H. 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.
10. Jang, W. H., Kim, S. J., Jeong, Y. J., Jun, H. J., Moon, I. S. and Seog, D. H. 2012. APP tail 1 (PAT1) interacts with Kinesin light chains (KLCs) through the tetratricopeptide repeat (TPR) domain. *J. Life Sci.* **22**, 1608-1613.
11. Kanai, Y., Dohmae, N. and Hirokawa, N. 2004. Kinesin transports RNA: isolation and characterization of an RNA-transporting granule. *Neuron* **43**, 513-525.
12. Miki, H., Miura, K. and Takenawa, T. 1996. N-WASP, a novel actin-depolymerizing protein, regulates the cortical cytoskeletal rearrangement in a PIP2-dependent manner downstream of tyrosine kinases. *EMBO J.* **15**, 5326-5335.
13. Millard, T. H., Sharp, S. J. and Machesky, L. M. 2004. Signalling to actin assembly via the WASP (Wiskott-Aldrich syndrome protein)-family proteins and the Arp2/3 complex. *Biochem. J.* **380**, 1-17.
14. 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.
15. 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.
16. Soderling, S. H., Binns, K. L., Wayman, G. A., Davee, S. M., Ong, S. H., Pawson, T. and Scott, J. D. 2002. The WRP component of the WAVE-1 complex attenuates Rac-mediated signalling. *Nat. Cell Biol.* **4**, 970-975.
17. Sossey-Alaoui, K. 2013. Surfing the big WAVE: insights into the role of WAVE3 as a driving force in cancer progression and metastasis. *Semin. Cell Dev. Biol.* **24**, 287-297.
18. Suetsugu, S., Miki, H. and Takenawa, T. 1999. Identification of two human WAVE/SCAR homologues as general actin regulatory molecules which associate with the Arp2/3 complex. *Biochem. Biophys. Res. Commun.* **260**, 296-302.
19. Takahashi, K. and Suzuki, K. 2008. Requirement of kinesin-mediated membrane transport of WAVE2 along microtubules for lamellipodia formation promoted by hepatocyte growth factor. *Exp. Cell Res.* **314**, 2313-2322.
20. Takenawa, T. and Suetsugu, S. 2007. The WASP-WAVE pro-

tein network: connecting the membrane to the cytoskeleton. *Nat. Rev. Mol. Cell Biol.* **8**, 37-48.
21. Wilson, M. H. and Holzbaur, E. L. 2015. Nesprins anchor kinesin-1 motors to the nucleus to drive nuclear distribution in muscle cells. *Development* **142**, 218-228.
22. Wobst, H., Schmitz, B., Schachner, M., Diestel, S., Leshchyns'ka, I. and Sytnyk, V. 2015. Kinesin-1 promotes post-Golgi trafficking of NCAM140 and NCAM180 to the cell surface. *J. Cell Sci.* **128**, 2816-2829.
23. Zhu, H., Lee, H. Y., Tong, Y., Hong, B. S., Kim, K. P., Shen, Y., Lim, K. J., Mackenzie, F., Tempel, W. and Park, H. W. 2012. Crystal structures of the tetratricopeptide repeat domains of kinesin light chains: insight into cargo recognition mechanisms. *PLoS One* **7**, e33943.

초록 : Kinesin Light Chain (KLC)의 Tetratricopeptide Repeat (TPR) 도메인을 통한 Scaffold 단백질 WAVE1과 Kinesin 1의 결합

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Kinesin superfamily proteins (KIFs)은 세포 내 소기관이나 단백질복합체를 미세소관을 따라 운반하는 모터단백질이다. Kinesin 1은 경쇄단위체(light chain subunit)를 통하여 결합함으로써 세포 내 소기관, 신경소포, 신경전달물질수용체, 신호전달단백질, mRNA 등 다양한 운반체를 운반하는 KIFs의 한 종류이다. Kinesin light chains (KLCs)은 모터기능이 없는 단위체로서 kinesin heavy chains (KHCs) 이량체와 결합하여 kinesin 1을 구성한다. KLCs은 여러 단백질과 결합하지만 아직 결합단백질이 충분히 밝혀지지 않았다. 본 연구에서 KLC1의 tetratricopeptide repeat (TPR) 영역과 결합하는 단백질을 분리하기 위하여 효모 two-hybrid 탐색을 수행한 결과 Wiskott-Aldrich syndrome의 원인단백질이며 액틴 세포골격 조절단백질인 WASP/WAVE family의 하나인 WAVE1을 분리하였다. WAVE1은 KLC1의 TPR 영역을 포함한 부위와 결합하지만 KHCs인 KIF5A, KIF5B, KIF5C와는 결합하지 않았다. 또한 KLC1은 WAVE1의 C-말단에 존재하는 verprolin/cofilin/acidic (VCA) 도메인과 결합하였으며, 다른 WAVE isoform인 WAVE2와 WAVE3과도 결합하였다. HEK-293T 세포에 WAVE1과 KLC1을 동시에 발현시켰을 때 두 단백질이 세포 내에서 같은 부위에 존재하며, WAVE1을 면역침강한 결과 KLC1뿐만 아니라 KIF5B가 같이 침강함을 확인하였다. 이러한 결과들은 kinesin 1이 WAVE 단백질복합체 혹은 WAVE로 덮여있는 운반체를 운반함을 시사한다.