Animal Cells and Systems 13: 9-15, 2009

Animal Cells
and
Systems
http://www.AnimalCells.or.kr

Identification of WDR7 as a Novel Downstream Target of the EphA8-Odin Signaling Complex

Eunjeong Park and Soochul Park*

Department of Biological Science, Sookmyung Women's University, Seoul 140-742, Korea

Abstract: Eph receptors and their ephrin ligands have been implicated in a variety of cellular processes such as cellular morphogenesis and motility. Our previous studies demonstrated that Odin, one of the Anks family proteins, functions as a scaffolding protein of the EphA8 signaling pathway leading to modulation of cell migration or axonal outgrowth. Here we show that WDR7 is associated with Odin and that it is possibly implicated in the EphA8 signaling pathway. WD40 repeats present in the COOH-terminal region of WDR7 appear to be crucial for its association with Odin, whereas the binding motif of Odin is located in between ankyrin repeats and PTB domain. Co-immunoprecipitation experiments revealed that association of WDR7 with Odin is enhanced by ephrin ligand treatment, possibly through forming large protein complexes including both EphA8 and ephrin-A5. Consistently, immunofluorescence staining experiments suggested that WDR7 constitute a component of the large protein complexes containing Odin, EphA8 and ephrin-A5. Taken together, our results suggest the WDR7-Odin complexes might be involved in the signaling pathway downstream of the EphA8 receptor.

Key words: Odin, Anks family protein, EphA8, WDR7

INTRODUCTION

The Eph family, the largest subfamily of receptor tyrosine kinases (RTKs), is comprised of at least sixteen different receptors. They bind to cell surface-attached ephrin ligands on the contacting cells, which have been identified up to ten different members to date. Eph-ephrin complexes can transduce bidirectional signals (in *trans*) which influence both the Eph receptor-expressing cells (forward signaling) and the ephrin-expressing cells (reverse signaling). Unlike other RTKs, Eph receptors do not regulate cell proliferation and survival (Kullander and Klein, 2002). Their unique bidirectional signaling mechanism has emerged as a key determinant of various developmental processes, including cardiovascular and skeletal development, axon guidance, synaptogenesis, and tissue patterning (Palmer and Klein, 2003).

A well-known effect of Eph signaling is retraction of the cell periphery following contact with ephrin-expressing cells (Pasquale, 2005). This repulsive event may be essential for axon guidance and sorting of Eph-expressing cells from ephrin-expressing cells during developmental process (Pasquale, 2008). Two different mechanisms can explain how Eph-ephrin-mediated adhesive response is turned into repulsion. One mechanism is to eliminate the Eph-ephrin complexes from the cell surface due to endocytosis of plasma membrane portions derived from both cells (Egea and Klein, 2007). The other mechanism is metalloprotease-mediated cleavage of ephrins (Egea and Klein, 2007; Himanen et al., 2007). In addition to how Eph receptors control repulsion versus attraction, the interplay between Eph signaling and various signaling molecules has been predicted to be a key factor in the control of its ultimate effects on cell behavior.

AβPP intracellular domain-associated protein 1b (AIDA-1b) and Odin (a deity in the Nordic mythology) belong to the ankyrin repeat and sterile alpha motif (SAM) domain containing (Anks) protein family. It has been known that AIDA-1b and Odin participate in modulating AβPP processing and inhibiting platelet-derived growth factor-mediated cell proliferation, respectively (Pandey et al., 2002; Ghersi et al., 2004). More recently, the Anks family proteins have been identified as a scaffolding protein which modulates the EphA8 signaling pathway related to cell motility and axonal retraction (Shin et al., 2007).

In the present study, we identified WD Repeat 7 (WDR7) as an adaptor protein that interacts with Odin through two WD40 repeats in its C-terminal portion. WD40 repeats are

*To whom correspondence should be addressed.
Tel: +82-2-710-9330; Fax: +82-2-715-9331
E-mail: scpark@sookmyung.ac.kr

conserved domains containing approximately 40-60 amino acids. They are initiated by a glycine-histidine (GH) dipeptide from the N terminus and end with a tryptophan-aspartic acid (WD) dipeptide at the C terminus. Between the GH and WD dipeptides is the conserved core sequence (Smith et al., 1999; Li and Roberts, 2001). In general, WD40 repeat proteins have been implicated in a wide spectrum of cellular processes, including RNA processing, transcriptional regulation (Williams et al., 1991; Hoey et al., 1993), mitotic spindle formation (de Hostos et al., 1991; Vaisman et al., 1995), regulation of vesicle formation and vesicular trafficking (Pryer et al., 1993), and control of cell division (Feldman et al., 1997). WDR7 does not have other known protein-protein interacting motifs or catalytic domains except for nine WD40 repeats. Although it has been implicated in Rab3A-mediated exocytosis of neurotransmitter through its interaction with Rab3 GDP/GTP exchange protein (Rab3 GEP), its function is largely unknown (Sanders et al., 2000; Nagano et al., 2002; Kawabe et al., 2003). Our current findings raised the possibility for the first time that WDR7-Odin complexes may play a critical role in the regulation of EphA8 receptor endocytosis.

MATERIALS AND METHODS

Yeast two-hybrid screen

The mouse Odin-ΔPTB (with a deletion of amino acids 865 to 1082 of mouse Odin) cloned into the pBHA vector (pEP11) was used as bait to screen a human fetal brain cDNA library consisting of 3.0×10^6 independent clones (Clontech). Briefly, yeast strain L40 [*MAT*a *his3200 trp1-901 lue2-3,112 ade2 (LYS2::lexAop)$_4$-HIS3 (URA3::lexAop)$_8$-lacZ GAL4*] was transformed with pEP11 and the human fetal brain cDNA library as described previously (Shin et al., 2007). The resulting transformants were screened for histidine prototrophy by using 1 mM 3-aminotriazole (3-AT) on selective medium lacking His, Trp, and Leu. Histidine-positive (His^+) clones were then assayed for blue coloring by colony lift with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). Among several His^+ $LacZ^+$ clones, five identical clones encompassing the COOH-terminal portion of WDR7 gene (GenBank accession no. NM-015285) were identified.

In vitro binding assay

Binding assays were performed as described previously (Gu et al., 2001). Purified glutathione *S*-transferase (GST)-tagged the COOH-terminal portion of WDR7 (GST-W7-C) protein (amino acids 1149 to 1490 of human WDR7) was immobilized onto glutathione-Sepharose 4B beads (GE Healthcare). The GST-W7-C immobilized beads were incubated with lysate prepared from 293T cells transfected with Odin in binding buffer (20 mM Tris-HCl (pH 7.5), 5 mM EDTA, 100mM NaCl, 0.1% Triton X-100) for 2 h at 4°C. After removal of the supernatant, the beads were washed three times with the same buffer, and bound proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Precipitated Odin was detected by immunoblotting with anti-Odin antibody.

Co-immunoprecipitation and Western blotting

Co-immunoprecipitation and Western blotting were performed as described previously (Gu et al., 2001). 293T cells were cotransfected with hemagglutinin (HA)-tagged WDR7 and Odin. After 24 h, transfected cells were harvested and cell lysates were prepared with protease inhibitors in PLC buffer (50 mM HEPES (pH 7.5), 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 10% glycerol, 100 mM NaF, 1 mM Na_3VO_4, and 1% Triton X-100). To purify target proteins, 1 μg of affinity-purified polyclonal antibody was incubated with precleared cell lysate for 1 h at 4°C and then precipitated with protein A-Sepharose (GE Healthcare) for 1 h at 4°C. The resulting proteins were resolved by SDS-PAGE and subjected to Western blot analysis by the indicated antibodies.

Immunofluorescence staining

Immunofluorescence staining was performed as described previously (Shin et al., 2007). 293 cells were stably transfected with WDR7 and EphA8. The indicated cells were transfected with EYFP-tagged Odin. The cells were stimulated with preclustered ephrinA5-Fc ligands or Fc control for 15 min and fixed with 4% paraformaldehyde-2% sucrose in phosphate-buffered saline for 20 min at room temperature, and rinsed three times for 5 min with TBST (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.1% Triton X-100). Cells were blocked with TBST containing 5% horse serum for 30 min at room temperature and then incubated with primary antibodies overnight at 4°C. After washing, cells were incubated with immunofluorescence-conjugated secondary antibodies for 1 h at room temperature. Photos were taken with a confocal microscope (model FV300; Olympus).

Antibodies

A polyclonal rabbit antibody specific for the JM domain of EphA8 was described previously (Choi et al., 1999). Anti-WDR7 antibodies were a gift from Dr. Yoshimi Takai. Anti-Odin antibodies were purchased from Calbiochem, anti-HA antibodies from Zymed, and tetramethyl rhodamine isocyanate-conjugated goat anti-rabbit IgG and fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG antibodies from Chemicon. Horseradish peroxidase-conjugated anti-rabbit IgG antibodies were purchased from Zymed.

RESULTS AND DISCUSSION

Fetal brain cDNA library screening identifies WDR7 as an Odin-interacting protein

Our previous studies indicated that Odin is a scaffolding protein to regulate the EphA8-mediated signaling pathway leading to cell migration and axonal retraction. Odin protein contains various conserved domains including ankyrin repeat, sterial alpha motif (SAM) and phosphotyrosine binding (PTB) domain. To further elucidate how Odin protein regulate the EphA8-mediated signaling pathway, we performed a yeast two-hybrid screen in which the Odin protein lacking its PTB domain (Odin-ΔPTB) was used as a bait to screen a human fetal brain cDNA library (Fig. 1A). We have identified five different clones as putative candidates, and one of them corresponds to the COOH-terminal portion of WDR7 protein. WDR7 is known to have nine WD40 repeats and the interacting clone, W7-C, contains two WD40 repeats present in its COOH-terminus (Fig. 1A).

As shown in Fig. 1B, we confirmed the association of interacting clone W7-C with the Odin-ΔPTB by transforming them back into yeast. In contrast, yeast transformants expressing either ankyrin repeats or SAM domains together with W7-C did not grow on His^{-} Trp^{-} Leu^{-} selective plates (Fig. 1B, left panel) and failed to show X-Gal staining on Trp^{-} Leu^{-} selective plates (Fig. 1B, right panel), suggesting that certain motif(s) existing in between ankyrin repeats and SAM domains is likely to be critical for interaction with WDR7.

In order to demonstrate a direct interaction between the Odin protein and W7-C, the W7-C interacting clone was expressed as a bacterial GST fusion protein and mixed with the lysates of 293T cells expressing Odin protein for the GST pull-down experiment. Transfection of Odin construct into 293T cells elevates the expression level of Odin at least by 5-fold as compared with that of the endogenously expressed Odin (data not shown). As expected, the GST fusion protein of W7-C, but not GST, was specifically co-precipitated with Odin protein not only from control cells but also from cells over-expressing Odin (Fig. 1C). These results indicate that the C-terminal region of WDR7 is sufficient to associate with the full-length Odin protein *in vitro*.

Next, we investigated whether the WD40 repeats in W7-C mediate its interaction with Odin. For this experiment, we constructed Xpress-tagged W7-C protein (W7-CΔWD) lacking two WD40 repeats (Fig. 1A), and this deletion mutant was co-expressed with the full-length Odin in 293T cells for co-immunoprecipitation experiments. As expected, the full-length Odin interacted strongly with W7-C (Fig. 1D, lane 4 from left) but failed to bind to W7-CΔWD (lane 8 from left). Taken together, these results suggest that WDR7 is able to interact with Odin through two WD40 repeats present in its C-terminal portion.

Treatment with ephrin ligand enhances the specific association between Odin and WDR7 proteins

Next, we examined whether a full-length WDR7 protein forms a stable complex with Odin protein in cultured cells. Expression vector encoding the full-length Odin protein was co-transfected into 293T cells together with a vector encoding HA-tagged WDR7 protein. Cell lysates were immunoprecipitated by anti-Odin antibody, followed by immunoblotting with anti-HA antibody. As shown in Fig. 2A, the WDR7 protein was readily detected in anti-Odin immunoprecipitates (lane 4). The robust co-immuno-precipitation of WDR7 with Odin was dependent on the expression of Odin (lanes 2 and 4), although nonspecific precipitation was also detectable in an immune complex which a rabbit IgG antibody replaced the anti-Odin antibody (lane 5). Consistent with these data, our co-immunoprecipitation study using anti-WDR7 antibody demonstrated that Odin was specifically co-precipitated with WDR7 from cell lysates (Fig. 2B, lane 4).

To further investigate whether an increased association between Odin and WDR7 requires the ephrin ligand stimulation, 293T cells transiently transfected with WDR7, Odin and EphA8 expression constructs were treated with preclustered ephrinA5-Fc ligands for 15 min, and cell lysates were subjected to an immunoprecipitation assay using anti-Odin antibody (Fig. 2C). Consistent with our previous report, EphA8 was co-immunoprecipitated with Odin in response to ephrinA5-Fc treatment (third panel, lane 2). Additionally, it was evident that the level of WDR7 protein co-immunoprecipitated with Odin was strongly elevated in response to preclustered ephrinA5-Fc, but not with Fc (first panel). Taken together, these findings suggest that association of WDR7 with Odin is enhanced by ephrin ligand treatment, suggesting a functional significance of WDR7-Odin complexes in EphA8-ephrin-A5 signaling.

WDR7 is co-localized with Odin and EphA8/ephrin-A5 complex

It has been previously shown that Odin is a cytoplasmic protein negatively acting downstream of the EGF signaling pathway (Pandey et al., 2002). WDR7 is also a cytoplasmic protein involved in vesicle trafficking, and our immuno-fluorescent staining using WDR7 antibody revealed that WDR7 is uniformly distributed in the cytoplasm of 293 cells (Fig. 3, fifth panels from left). Since both Odin and WDR7 antibodies were derived from rabbits, enhanced yellow fluorescent protein (EYFP)-tagged Odin construct was generated for co-localization study. Although Odin is predicted to be mainly present in the cytoplasm, EYFP-tagged Odin was observed in both cytoplasm and nucleus,

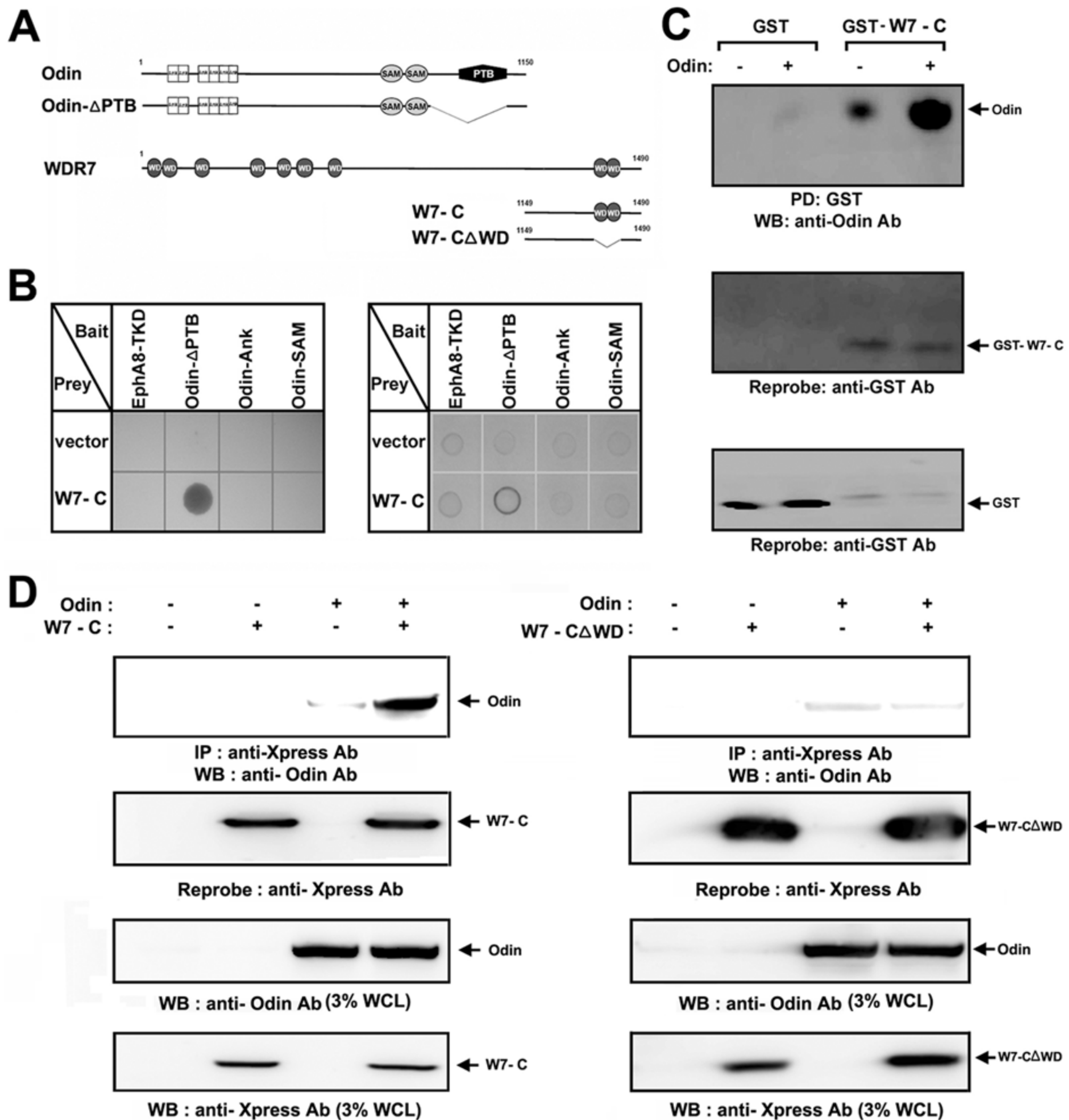

Fig. 1. Odin interacts with the WDR7 protein. (A) Domain structures of Odin, Odin-ΔPTB (with a deletion of amino acids 865 to 1082 of mouse Odin), WDR7, W7-C (amino acids 1149 to 1490 of human WDR7), and W7-CΔWD (with a deletion of amino acids 1351 to 1432 of W7-C). SAM, SAM domain; Ank, ankyrin repeat domain; PTB, PTB domain; WD, WD40 repeats domain. (B) Growth and X-Gal staining analysis indicated that the interacting clone, W7-C binds to Odin-ΔPTB. W7-C was co-expressed in the yeast two-hybrid assay with the LexA DNA binding domain alone or with LexA fusions to the Odin-ΔPTB, ankyrin repeat domain of Odin (Odin-Ank) and SAM domain of Odin (Odin-SAM). Yeast transformants were cultured on His^- Trp^- Leu^- selective plates in the presence of 1 mM 3-AT (left panel) and on Trp^- Leu^- selective plates in the absence of 3-AT prior to X-Gal staining using a filter-lifting method (right panel). Yeast cells transformed with the tyrosine kinase domain (TKD) of EphA8 as bait served as a negative control. Yeast transformants showing negative growth on His^- Trp^- Leu^- selective plates in the presence of 1 mM 3-AT failed to show X-Gal staining on Trp^- Leu^- selective plates. (C) Demonstration of the C-terminal region of WDR7 interaction with the full-length Odin protein. Purified proteins (GST or GST-W7-C) were mixed with whole-cell lysates from 293T cells transfected with the control vector (lanes 1 and 3) or an Odin expression construct (lanes 2 and 4). Bound proteins were pulled down using glutathione beads. The washed beads were separated by 10% SDS-PAGE and Western blotted (WB) using anti-Odin antibody as a probe (top panel). The same blot was stripped and reprobed with anti-GST antibody (middle and bottom panel). (D) Co-immunoprecipitation of W7-C and Odin in 293T cells (left panels). Cells were transfected with Xpress-tagged W7-C and Odin as indicated in each lane. Then total cell lysates were immunoprecipitated with anti-Xpress antibody and subjected to Western blotting with anti-Odin (first panel). The same blot was stripped and reprobed with anti-Xpress antibody (second panel). A sample (3%) of each total cell lysate was analyzed directly by Western blotting using anti-Odin antibody and anti-Xpress antibody (third and fourth panel). Co-immunoprecipitation of W7-CΔWD and Odin in 293T cells (right panels). Experiments were performed essentially as described for left panels, except that W7-CΔWD was used. Experiments shown in panel D were performed at the same time. PD, Pulldown; WB, Western blot; WCL, whole-cell lysate.

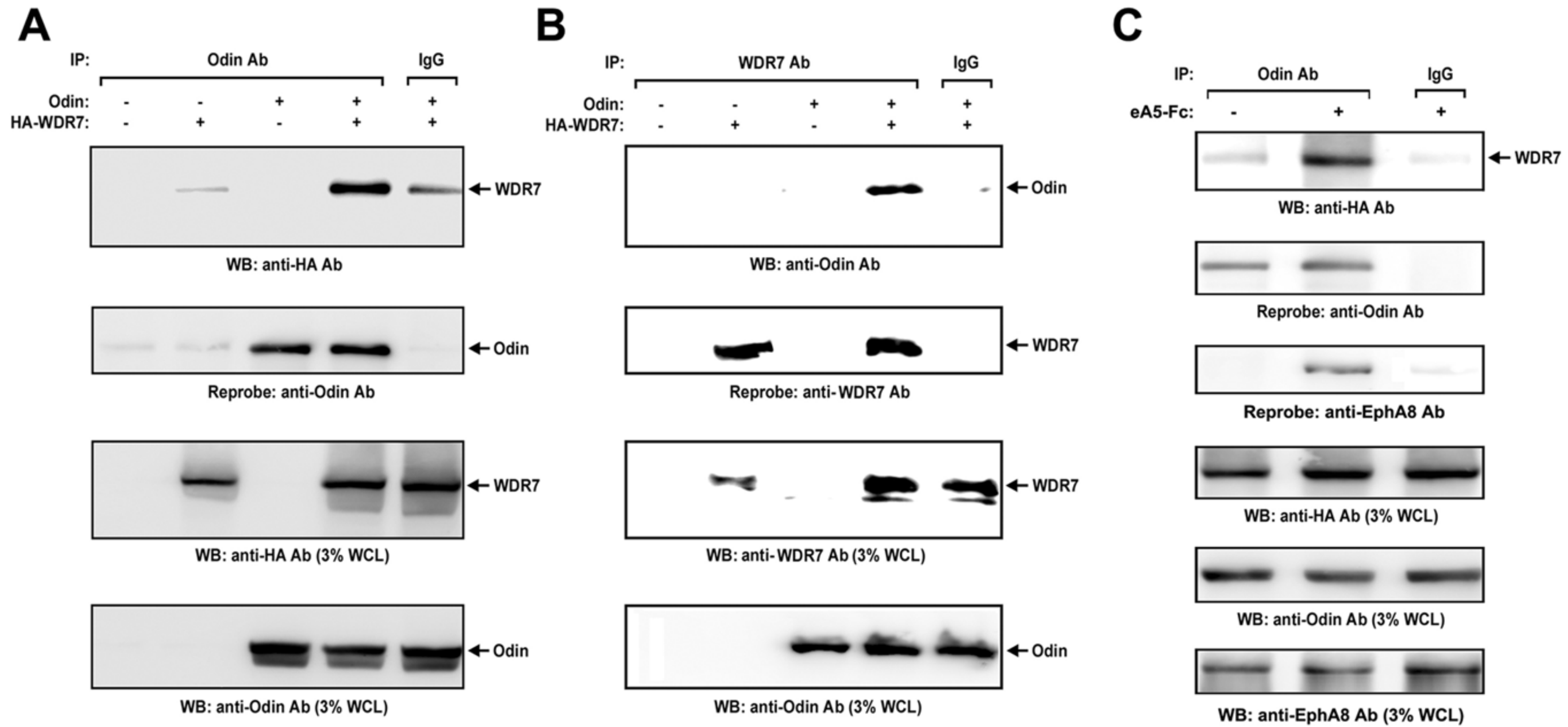

Fig. 2. EphrinA5 stimulation enhances the specific complex formation of Odin with WDR7 proteins. (A) Co-immunoprecipitation of WDR7 and Odin in 293T cells. Cells were transfected with HA-tagged WDR7 and Odin as indicated in each lane, and then total cell lysates were immunoprecipitated with anti-Odin antibody, followed by immunoblotting with anti-HA antibody (first panel). The same blot was stripped and reprobed with anti-Odin antibody (second panel). A sample of each total cell lysate was analyzed directly by Western blotting using anti-HA antibody and anti-Odin antibody (third and fourth panel). (B) Reciprocal co-immunoprecipitation of WDR7 and Odin in 293T cells. Experiments were performed essentially as described for panel A, except that reciprocal antibodies were used, as indicated in each panel. (C) 293T cells were transiently transfected with WDR7, Odin and EphA8 expression constructs. After 24 h, cells were stimulated with preclustered ephrinA5-Fc ligands for 15 min. Total cell lysates were immunoprecipitated with anti-Odin antibody or control IgG and then immunoblotted with anti-HA antibody (first panel). The same blot was stripped and reprobed with anti-Odin antibody and anti-EphA8 antibody (second and third panel). A sample of each total cell lysate was analyzed directly by Western blotting using anti-HA antibody, anti-Odin antibody and anti-EphA8 antibody (fourth, fifth and sixth panel). IP, Immunoprecipitation; WB, Western blot; WCL, whole-cell lysate.

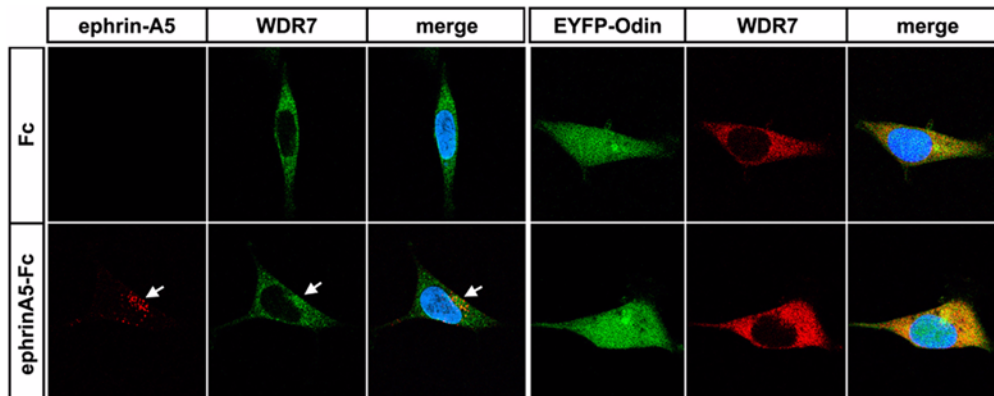

Fig. 3. WDR7 is co-localized with Odin and EphA8/ephrin-A5 complex in the cytoplasmic region. 293 cells were stably transfected with WDR7 and EphA8 receptor. The cells were stimulated with preclustered ephrinA5-Fc ligands or Fc control for 15 min and stained with anti-WDR7 antibody (first panels, second panels and third panels from left). The indicated cells were transfected with EYFP-tagged Odin. The cells were stimulated and stained with as described for previously (fourth panels, fifth panels and sixth panels from left).

suggesting that Odin may be translocated from the cytoplasm to the nucleus in the presence of certain biochemical stimulation. Nevertheless, the cytoplasmic EYFP-tagged Odin appears to be co-localized well with WDR7, irrespective of ephrin-A5 treatment (Fig. 3, sixth panels from left). Since both Odin and WDR7 are uniformly distributed possibly as the same protein complex in the cytoplasm, it seems difficult to demonstrate a clear-cut and enhanced co-localization of these two proteins in response to ephrin-A5 stimulation. To further investigate whether WDR7 is detectable in the ephrinA5/EphA8 protein complexes, cells over-expressing EphA8 were treated with preclustered ephrinA5-Fc for 15 min. During this treatment, the ephrin-A5/EphA8 complexes were rapidly internalized into the cytoplasm (Fig. 3, first panels from left) and these complexes were partially co-localized with WDR7 (third panels from left), just like Odin as previously demonstrated. Taken together, our results suggest that WDR7 may constitute a component of the large protein complexes containing Odin, EphA8 and ephrin-A5.

In this report, we have determined that WDR7 protein interacts with Odin. These findings suggest that the WDR7-Odin complexes have a high probability to play a role in the signaling pathway downstream of the EphA8 receptor in response to ephrin-A. WDR7 has been known to bind both Rab3 GEP and GAP, possibly suggesting that it function in Ca^{2+}-dependent exocytosis of neurotransmitter (Nagano et al., 2002; Kawabe et al., 2003). However, it has not been yet determined that WDR7 affects Rab3 GEP or GAP activity. Nevertheless, our findings described in this report suggest that the scaffolding proteins such as Odin and WDR7 may provide a molecular linkage between EphA receptors and the Rab3 small G proteins. This molecular linkage may be crucial for the regulation of Ca^{2+}-dependent exocytosis of neurotransmitter in the presynaptic axon terminal, which should be determined in our future work.

ACKNOWLEDGMENTS

We thank Dr. Yoshimi Takai for providing the WDR7 construct and its antibody. This work was supported by Sookmyung Women's University in 2007.

REFERENCES

Choi SA and Park SC (1999) Phosphorylation at Tyr-838 in the kinase domain of EphA8 modulates Fyn binding to the Tyr-615 site by enhancing tyrosine kinase activity. *Oncogene* 18: 5413-5422.

de Hostos EL, Bradtke B, Lottspeich F, Guggenheim R, and Gerisch G (1991) Coronin, an actin binding protein of *Dictyostelium discoideum* localized to cell surface projections, has sequence similarities to G protein beta subunits. *EMBO J* 10: 4097-4104.

Egea J and Klein R (2007) Bidirectional Eph-ephrin signaling during axon guidance. *Trends Cell Biol* 17: 230-238.

Feldman RM, Correll CC, Kaplan KB, and Deshaies RJ (1997) A complex of Cdc4p, Skp1p, and Cdc53p/cullin catalyzes ubiquitination of the phosphorylated CDK inhibitor Sic1p. *Cell* 91: 211-230.

Ghersi E, Noviello C, and D'Adamio L (2004) Amyloid-β protein precursor (AβPP) intracellular domain-associated protein-1 proteins bind to AβPP and modulate its processing in an isoform-specific manner. *J Biol Chem* 279: 49105-40112.

Gu CG and Park SC (2001) The EphA8 receptor regulates integrin activity through p110γ phosphatidylinositol-3 kinase in a tyrosine kinase activity-independent manner. *Mol Cell Biol* 21: 4579-4597.

Himanen J-P, Saha N, and Nikolov DB (2007) Cell-cell signaling via Eph receptors and ephrins. *Curr Opin Cell Biol* 19: 534-542.

Hoey T, Weinzierl RO, Gill G, Chen JL, Dynlacht BD, and Tjian R (1993) Molecular cloning and functional analysis of Drosophila TAF110 reveal properties expected of coactivators. *Cell* 72: 247-260.

Kawabe H, Sakisaka T, Yasumi M, Shingai T, Izumi G, Nagano F, Deguchi-Tawarada M, Takeuchi M, Nakanishi H, and Takai Y

(2003) A novel rabconnectin-3-binding protein that directly binds a GDP/GTP exchange protein for Rab3A small G protein implicated in Ca^{2+}-dependent exocytosis of neurotransmitter. *Genes Cells* 8: 537-546.

Kullander K and Klein R (2002) Mechanisms and functions of Eph and ephrin signalling. *Nat Rev Mol Cell Biol* 3: 475-486.

Li D and Roberts R (2001) WD-repeat proteins: structure characteristics, biological function, and their involvement in human diseases. *Cell Mol Life Sci* 58: 2085-2097.

Nagano F, Kawabe H, Nakanishi H, Shinohara M, Deguchi-Tawarada M, Takeuchi M, Sasaki T, and Takai Y (2002) Rabconnectin-3, a novel protein that binds both GDP/GTP exchange protein and GTPase-activating protein for Rab3 small G protein family. *J Biol Chem* 277: 9629-9632.

Palmer A and Klein R (2003) Multiple roles of ephrins in morphogenesis, neuronal networking, and brain function. *Genes Dev* 17: 1429-1450.

Pandey A, Blagoev B, Kratchmarova I, Fernandez M, Nielsen M, Kristiansen TZ, Ohara O, Podtelejnikov AV, Roche S, Lodish HF, and Mann M (2002) Cloning of a novel phosphotyrosine binding domain containing molecule, Odin, involved in signaling by receptor tyrosine kinases. *Oncogene* 21: 8029-8036.

Pasquale EB (2005) Eph receptor signalling casts a wide net on cell behaviour. *Nat Rev Mol Cell Biol* 6: 462-475.

Pasquale EB (2008) Eph-ephrin bidirectional signaling in physiology and disease. *Cell* 133: 38-52.

Pryer NK, Salama NR, Schekman R, and Kaiser CA (1993) Cytosolic Sec13p complex is required for vesicle formation from the endoplasmic reticulum in vitro. *J Cell Biol* 120: 865-875.

Sanders S, Keck-Waggoner CL, Zimonjic DB, Popescu NC, and Thorgeirsson SS (2000) Assignment of WDR7 (alias TRAG, TGF-beta resistance associated gene) to orthologous regions of human chromosome 18q21.1→q22 and mouse chromosome 18D.1-E.3 by fluorescence in situ hybridization. *Cytogenet Cell Genet* 88: 324-325.

Shin JD, Gu CG, Park EJ, and Park SC (2007) Identification of phosphotyrosine binding domain-containing proteins as novel downstream targets of the EphA8 signaling function. *Mol Cell Biol* 27: 8113-8126.

Smith TF, Gaitatzes C, Saxena K, and Neer EJ (1999) The WD repeat: a common architecture for diverse functions. *Trends Biochem Sci* 24: 181-185.

Vaisman N, Tsouladze A, Robzyk K, Ben-Yehuda S, Kupiec M, and Kassir Y (1995) The role of *Saccharomyces cerevisiae* Cdc40p in DNA replication and mitotic spindle formation and/or maintenance. *Mol Gen Genet* 247: 123-136.

van der Voorn L, and Ploegh HL (1992) The WD-40 repeat. *FEBS Lett* 307: 131-134.

Williams FE, Varanasi U, and Trumbly RJ (1991) The CYC8 and TUP1 proteins involved in glucose repression in *Saccharomyces cerevisiae* are associated in a protein complex. *Mol Cell Biol* 11: 3307-3316.

[Received November 3, 2008; accepted December 11, 2008]