Biochemical and Biophysical Research Communications 442 (2013) 16–21

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Proteomic analysis of Girdin-interacting proteins in migrating new neurons in the postnatal mouse brain

Haruko Ota [a,b], Takao Hikita [a], Tomoki Nishioka [c], Mami Matsumoto [a], Jun Ito [a], Naoya Asai [d], Atsushi Enomoto [d], Masahide Takahashi [d], Kozo Kaibuchi [c], Kazuya Sobue [b], Kazunobu Sawamoto [a,*]

[a] Department of Developmental and Regenerative Biology, Nagoya City University, Graduate School of Medical Sciences, Aichi, Japan
[b] Department of Anesthesiology and Medical Crisis Management, Nagoya City University, Graduate School of Medical Sciences, Aichi, Japan
[c] Department of Cell Pharmacology, Nagoya University, Graduate School of Medicine, Aichi, Japan
[d] Department of Pathology, Nagoya University, Graduate School of Medicine, Aichi, Japan

ARTICLE INFO

Article history:
Received 3 October 2013
Available online 6 November 2013

Keywords:
Neuronal migration
Postnatal neurogenesis
Ventricular–subventricular zone (V–SVZ)
Liquid chromatography tandem mass spectrometry (LC–MS/MS)
Girdin

ABSTRACT

Neural stem cells continuously generate new neurons in the ventricular–subventricular zone (V–SVZ) of the postnatal and adult mammalian brain. New neurons born in the rodent V–SVZ migrate toward the olfactory bulb (OB), where they differentiate into interneurons. To reveal novel intracellular molecular mechanisms that control postnatal neuronal migration, we performed a global proteomic search for proteins interacting with Girdin, an essential protein for postnatal neuronal migration. Using GST pull-down and LC–MS/MS shotgun analysis, we identified cytoskeletal proteins, cytoskeleton-binding proteins, and signal-transduction proteins as possible participants in neuronal migration. Our results suggest that Girdin and Girdin-interacting proteins control neuronal migration by regulating actin and/or microtubule dynamics.

1. Introduction

Neuronal migration occurs in the brain of postnatal and adult animals, in addition to embryos [1–4]. In most vertebrate brains including those of mammals, new neurons are constantly generated throughout life in two regions: the subgranular zone of the dentate gyrus in the hippocampus and the ventricular–subventricular zone (V–SVZ) of the lateral ventricles. New neurons originating in the V–SVZ migrate through the rostral migratory stream (RMS) toward the olfactory bulb (OB) [5–9] and mature into two distinct types of local olfactory interneurons: granule cells and periglomerular cells [8,10]. These new neurons are reported to join the existing neural circuitry in the OB and to have olfactory functions [11–13]. The migration of new neurons from the V–SVZ to OB has several unique characteristics. In the RMS, the new neurons form aggregates that migrate tangentially over a long distance [14,15]. Once they reach the OB, they migrate radially to their final positions. The molecular mechanisms underlying this unique neuronal migration process are not fully understood.

We recently reported that neuronal migration in the RMS is disrupted in knockout mice of the gene encoding Girdin [16], an actin cytoskeleton-binding protein originally identified as a substrate of the serine/threonine kinase Akt1 [17]. Girdin is a large, 1870-amino acid protein with a predicted molecular mass of 216 kDa and unique amino (NT) and carboxy-terminal (CT) domains flanking a long coiled-coil domain. Previous studies indicated that the NT and CT domains are involved in protein–protein interactions that regulate Girdin's oligomerization and remodeling of the actin cytoskeleton [17]. Thus, the identification of novel molecules that interact with these domains should increase our understanding of the mechanisms by which Girdin controls the cytoskeleton in migrating new neurons.

To search for novel intracellular proteins that control neuronal migration from the V–SVZ to the OB in the postnatal brain, we performed a proteomic screen for Girdin-interacting proteins in the V–SVZ. We performed glutathione-S transferase (GST) pull-down using a purified GST-Girdin fusion protein and liquid chromatography tandem mass spectrometry (LC–MS/MS) shotgun analysis. From these data, we identified putative regulators of neuronal migration, including cytoskeletal proteins, cytoskeleton binding proteins, and signal transduction proteins. Further investigation of these candidate Girdin-interacting proteins may help clarify the mechanisms that control neuronal migration from the V–SVZ to the OB.

* Corresponding author. Address: Department of Developmental and Regenerative Biology, Nagoya City University, Graduate School of Medical Sciences, 1-Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan. Fax: +81 52 851 1898.
E-mail address: sawamoto@med.nagoya-cu.ac.jp (K. Sawamoto).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.10.126

2. Materials and methods

2.1. Animals

Wild-type (WT) Institute of Cancer Research (ICR) mice and Wistar rats were purchased from SLC (Shizuoka, Japan). All the animal experimental procedures complied with national regulations and guidelines, were reviewed by the Institutional Laboratory Animal Care and Use Committee, and were approved by the President of Nagoya City University.

2.2. Antibodies

The antibodies used in this study included sheep anti-Girdin polyclonal (R&D Systems, Minneapolis, MN), goat anti-Dcx polyclonal (Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti-βIII-tubulin (Sigma, St Louis, MO), and rat anti-GFP (Roche, Basel, Switzerland) antibodies, and normal sheep IgG (Santa Cruz Biotechnology). Anti-CLASP2 antibody was described previously[18].

2.3. Preparation of recombinant GST–Girdin fusion proteins

The construction of Girdin fragment (NT, M1, M2, CT, CT1, and CT2) expression vectors was previously described [19]. Recombinant GST, GST-NT, and GST-CT2 proteins were affinity purified using Glutathione Sepharose 4B beads (GE Healthcare, Piscataway, NJ), according to the manufacturer's protocol. To confirm the protein purification, aliquots of the purified proteins were subjected to SDS–polyacrylamide gel electrophoresis (PAGE), followed by Coomassie Brilliant Blue (CBB) staining.

2.4. Affinity purification of Girdin-interacting proteins

The V–SVZ tissue was dissected from postnatal-day (P) 2–4 wild-type mice and homogenized in lysis buffer [50 mM Tris–HCl (pH 8.0), 100 mM NaCl, 1 mM EDTA, 1% NP-40, 10 μg/ml leupeptin, 1 mM dithiothreitol]. The lysates were briefly sonicated and cleared by centrifugation at 20,000*g* for 10 min at 4 °C. One nanomole of purified GST or GST-fusion Girdin proteins (GST-NT, GST-CT2) was incubated with Glutathione Sepharose 4B beads (GE Healthcare) for 1 h at 4 °C and washed with lysis buffer. The resulting GST-, GST-NT-, or GST-CT2-coated beads were incubated with the supernatant of the spun V–SVZ lysates for 1 h at 4 °C. The beads were washed extensively with washing buffer [20 mM Tris–HCl (pH 8.0), 1 mM EDTA, 1 mM DTT, 100 mM NaCl], then the purified proteins and their binding proteins were eluted with 1 M NaCl buffer [20 mM Tris–HCl (pH 8.0), 1 mM EDTA, 1 mM DTT, 1 M NaCl] and GSH buffer [20 mM Tris–HCl (pH 8.0), 1 mM EDTA, 1 mM DTT, 10 mM Glutathione]. Aliquots of the eluates were subjected to SDS–PAGE, followed by silver staining.

2.5. Mass spectrometry

The 1 M NaCl eluates of the GST pull-down samples were dialyzed against 20 mM Tris–HCl, pH 8.0; 1 mM EDTA, 1 mM DTT. The proteins in the eluates were digested by trypsin for 16 h at 37 °C after reduction, alkylation, demineralization, and concentration. Nanoelectrospray tandem mass analysis was performed using an LTQ Orbitrap XL (Thermo Fisher Scientific, Waltham, MA) system connected to an HTC-PAL autosampler and a Paradigm MS4 HPLC (Bruker-Michrom, Auburn, CA) with a C18 reversed-phase column and Michrom's ADVANCE Plug and Play Nano Source (Bruker-Michrom). Reversed-phase chromatography was performed with a linear gradient (0 min, 5% B; 45 min, 100% B) of solvent A (2% acetonitrile with 0.1% trifluoroacetic acid) and solvent B (98% acetonitrile with 0.1% trifluoroacetic acid) at an estimated flow rate of 500 nl/min. A precursor ion scan was carried out using a 396–2000 mass to charge ratio (m/z) prior to MS/MS analysis. Multiple MS/MS spectra were submitted to the Mascot program (Matrix Science, Boston, MA) to search for MS/MS ions.

2.6. Criteria for protein identification

The Scaffold program (version Scaffold_4.0.5, Proteome Software, Portland, OR) was used to validate the MS/MS-based peptide and protein identifications. Peptide identifications were accepted if they could be established at a probability greater than 95.0%. The peptide probabilities obtained from Mascot were assigned by the Scaffold Local FDR algorithm. The peptide probabilities from X! Tandem were assigned by the Peptide Prophet algorithm [20] with Scaffold delta-mass correction. Protein identifications were accepted if they could be established at a probability greater than 95.0% and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm [21]. Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principle of parsimony. Proteins sharing significant peptide evidence were annotated with GO terms from gene_association.goa_uniprot [22].

2.7. Immunohistochemistry and microscopy

Brains were fixed by transcardiac perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer, postfixed overnight in the same fixative, and cut into 60-μm-thick floating coronal or sagittal sections using a vibratome (VT1200S, Leica, Heidelberg, Germany) as reported previously [14,23]. For immunostaining, the sections were incubated for 1 h in blocking solution (10% donkey serum and 0.2% Triton X-100 in PBS) overnight at 4 °C with the primary antibodies, and for 2 h at room temperature with Alexa Fluor-conjugated secondary antibodies (Life technologies, Carlsbad, CA). Signal amplification was performed with biotinylated secondary antibodies (Jackson Laboratory, West Grove, PA) and the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA), and the signals were visualized using the TSA Fluorescence System (PerkinElmer, Waltham, MA). Nuclei were stained with Hoechst 33342 (Sigma). Confocal images were obtained using an LSM700 laser-scanning microscope system (Zeiss, Oberkochen, Germany).

2.8. Culture of V–SVZ cells and immunocytochemistry

The V–SVZ was dissected from P1–4 wild-type mice in L-15 medium and dissociated with trypsin–EDTA (Life technologies). The cells were spread onto cover glasses coated with poly-L-lysine (Sigma) and laminin (Life technologies) at a density of 2.5×10^5 cells per 1.88 square centimeters, then cultured in neurobasal medium containing 10% fetal bovine serum (FBS), 50 U/ml penicillin–streptomycin, 2 mM L-glutamine, and 2% B27 (Life technologies) supplement for 48 h. The cells were fixed with 4% paraformaldehyde. For immunostaining, the cells were incubated for 5 min in 0.3% Triton X-100 in PBS, 30 min in blocking solution (10% donkey serum and 0.03% Tween-20 in PBS), overnight at 4 °C with the primary antibodies, and for 2 h at room temperature with Alexa Fluor-conjugated secondary antibodies (Life technologies).

2.9. GST pull-down assay and Western blot analysis

The construction of CLASP2 fragment (N1, N2, M, and C) expression vectors was previously described [18]. COS7 cells were transfected with various combinations of expression vectors including each domain of CLASP2 tagged with GFP at the N terminus, and the CT domain of Girdin tagged with GST at the N terminus, and cultured for 24 h. The cells were then lysed with lysis buffer, sonicated briefly, and clarified by centrifugation at 20,000*g* for 10 min at 4 °C. The supernatant was then incubated with Glutathione Sepharose 4B beads (GE Healthcare) for 6–8 h at 4 °C. The beads were then washed extensively with the washing buffer, and the purified proteins and their binding proteins were eluted with SDS sample dilution buffer [125 mM Tris–HCl (pH 6.8), 2% SDS, 10 μ/ml bromophenol blue, 80 mM DTT]. For western blot analyses, aliquots of the eluates were separated by SDS–PAGE. Proteins were transferred to PVDF membranes, blocked in 0.5% skim milk in TBS [50 mM Tris/HCl (pH 7.4), 138 mM NaCl, 2.7 mM KCl] containing 0.01% Tween 20, incubated with primary antibodies, and detected by horseradish peroxidase-conjugated secondary antibodies (Dako) with enhanced luminal-based chemiluminescent (ECL) western blotting detection reagent (GE Healthcare). Signals were detected and measured with a cooled charge-coupled device camera (LAS 3000mini; Fujifilm, Tokyo, Japan).

3. Results

We performed a global search for Girdin-interacting proteins in the V–SVZ using GST pull-down and LC–MS/MS shotgun analysis. First, to prepare baits for the pull-down, each domain of Girdin tagged with GST at its N terminus was expressed in *Escherichia coli*. Girdin has unique amino-terminal (NT) and carboxy-terminal (CT1 and CT2) domains flanking a long coiled-coil domain (M1 and M2) (Fig. 1A) [17,19]. Of these GST-fused domains, only GST-NT and GST-CT2 could be efficiently solubilized and affinity purified like GST alone (Fig. 1B), suggesting that these two domains are highly hydrophilic.

Next, to isolate and concentrate the Girdin-interacting proteins, GST pull-down was performed using Glutathione Sepharose 4B beads conjugated with GST-NT, GST-CT2, or GST (as a control). The beads were incubated with the lysate of postnatal mouse V–SVZ, then washed, and the bound proteins were eluted with 1 M NaCl. Aliquots of the eluate were then subjected to SDS–PAGE, followed by silver staining (Fig. 2). Many additional bands were observed in the 1 M NaCl eluate from GST-CT2 but not GST-NT, compared with the purified bait proteins. Therefore, we focused on the GST-CT2-interacting proteins.

To identify the proteins that interacted with the CT2 of Girdin, the 1 M NaCl eluates of the GST and GST-CT2 pull-down were subjected to LC–MS/MS shotgun analysis. We identified 198 proteins that were in the sample eluted from GST-CT2 but not GST (>2 identified peptides per protein, probability > 95%). These candidate proteins were then categorized by the functional criteria defined by the Gene Ontology Consortium (GO; The Gene Ontology Consortium, 2000; www.geneontology.org) [22]. To select for proteins that were most likely to be related to neuronal migration, we screened for candidates with the particular GO terms 'locomotion' and/or 'cytoskeleton'. Consequently, 7 molecules related to 'locomotion' and 25 molecules related to 'cytoskeleton'were identified. Proteins categorized with both of these GO terms were CLASP2, MARK1, Junction plakoglobin, and Tubulin-beta 2B (Table 1).

Of these proteins, we chose the microtubule plus-end tracking protein CLASP2, a protein involved in microtubule stabilization and cell migration [18,24] as a representative candidate Girdin-interacting protein for further characterization. To study the expression pattern of CLASP2, we stained wild-type mouse brain sections with an anti-CLASP2 antibody combined with antibodies against Girdin and doublecortin (Dcx), a cell-specific marker for new neurons. CLASP2 and Girdin were detected in almost all the Dcx-expressing cells in the V–SVZ (Fig. 3A). To determine the subcellular localization of CLASP2, new neurons dissociated from postnatal mouse V–SVZ tissue were stained for CLASP2, Girdin, and βIII-tubulin. Both CLASP2 and Girdin were detected at the proximal leading process of new neurons, suggesting that these proteins are co-localized in new neurons (Fig. 3B). To determine the region of CLASP2 that binds to Girdin CT, we performed GST pull-down assays using lysates from COS cells that exogenously expressed each

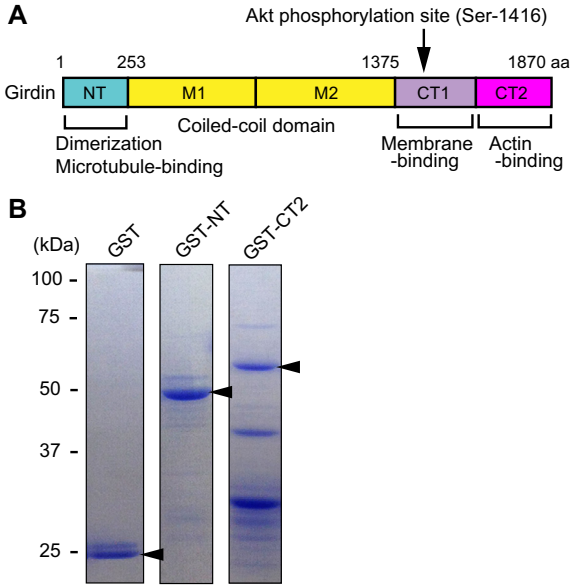

Fig. 1. Purification of GST fusion proteins. (A) Proposed functional domain structures of the human Girdin protein. (B) GST, GST-NT, and GST-CT2 proteins were affinity-purified and analyzed by SDS–PAGE, followed by CBB staining. Arrowheads indicate the main bands of the purified proteins. GST-CT2 was partially degraded.

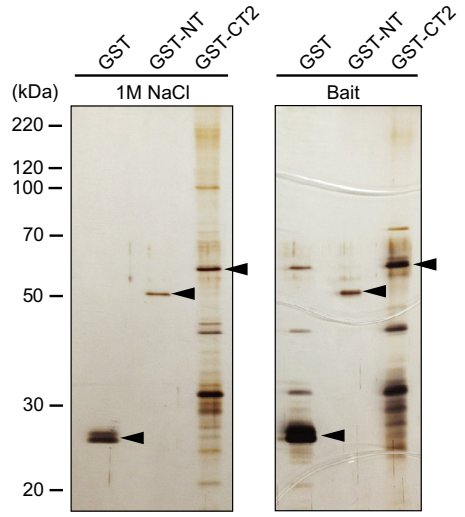

Fig. 2. Isolation of Girdin-interacting proteins. GST pull-down of V–SVZ lysates in the postnatal mouse brain was performed using GST- or GST-fusion Girdin protein-coated beads. Aliquots of the 1 M NaCl eluates (left) and each bait protein (right) were subjected to SDS–PAGE, followed by silver staining. The arrowheads indicate bait proteins.

Table 1
Identified Girdin-interacting molecules obtained with the 'locomotion' and/or 'cytoskeleton' criteria defined by the Gene Ontology Consortium (GST-CT2).

Protein name	Gene symbol	Accession number	MW (kDa)	Allen brain atlas	Locomotion	Cytoskeleton
40S ribosomal protein S7	Rps7	RS7_MOUSE	22			Cytoskeleton
Actin, cytoplasmic		ACTC_BRABE	42			Cytoskeleton
Actin-1	ACTA	ACT1_PHYIN	42	V–SVZ, RMS		Cytoskeleton
Calcium/calmodulin-dependent protein kinase type II subunit beta	Camk2b	KCC2B_MOUSE	60			Spindle midzone
CAP-Gly domain-containing linker protein 2	Clip2	CLIP2_MOUSE	116			Microtubule
CLIP-associating protein 2	Clasp2	CLAP2_MOUSE	141		Cell migration	Cytoskeleton
Desmoplakin	Dsp	DESP_MOUSE	333			Cornified envelope
Drebrin	Dbn1	DREB_MOUSE	77	V–SVZ, RMS		Actin cytoskeleton
ERC protein 2	Erc2	ERC2_MOUSE	111			Cytoskeleton
Ezrin	Ezr	EZRI_MOUSE	69			Actin cytoskeleton
Girdin	CCDC88A	GRDN_HUMAN	216	V–SVZ, RMS	Cell migration	
Girdin	Ccdc88a	GRDN_MOUSE	216	V–SVZ, RMS	Cell migration	
Guanine nucleotide-binding protein subunit beta-2-like 1	Gnb2l1	GBLP_MOUSE	35	V–SVZ, RMS		Cytoskeleton
Heterogeneous nuclear ribonucleoprotein H	Hnrnph1	HNRH1_MOUSE	49	V–SVZ		Actin cytoskeleton
Histone H4	Hist1h4a	H4_MOUSE	11			Actin cytoskeleton
Junction plakoglobin	Jup	PLAK_MOUSE	82		Cell migration	Cytoskeleton
Keratin, type I cytoskeletal 16	Krt16	K1C16_MOUSE	52			Intermediate filament
Keratin, type II cytoskeletal 3	KRT3	K2C3_RABIT	64			Intermediate filament
Keratin, type II cytoskeletal 73	Krt73	K2C73_MOUSE	59			Intermediate filament
Kinesin-like protein KIF2A	Kif2a	KIF2A_MOUSE	80			Spindle pole
Microtubule-associated protein 2	Map2	MAP2_MOUSE	199			Microtubule
Nucleophosmin	Npm1	NPM_MOUSE	33			Cytoskeleton
Protein RCC2	Rcc2	RCC2_MOUSE	56	V–SVZ, RMS		Spindle
Serine/threonine-protein kinase DCLK1	Dclk1	DCLK1_MOUSE	84		Neuron migration	
Serine/threonine-protein kinase MARK1	Mark1	MARK1_MOUSE	88		Neuron migration	Cytoskeleton
Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform	Ppp2ca	PP2AA_MOUSE	36			Cytoskeleton
Spectrin alpha chain, non-erythrocytic 1	Sptan1	SPTN1_MOUSE	285			Cortical cytoskeleton
Spindlin-1	Spin1	SPIN1_MOUSE	30			Spindle
Tubulin alpha chain	Tuba	TBA_XENLA	50	V–SVZ, RMS		Microtuble
Tubulin alpha-1B chain	TUB-1B	TBAB_SCHCO	49	V–SVZ, RMS		Microtuble
Tubulin beta-2A chain	Tubb2a	TBB2A_MOUSE	50	V–SVZ, RMS		Microtuble
Tubulin beta-2B chain	Tubb2b	TBB2B_MOUSE	50	V–SVZ, RMS	Neuron migration	Microtuble
Tubulin beta-3 chain	Tubb3	TBB3_MOUSE	50	V–SVZ		Microtuble cytoskeleton
UPF0568 protein C14orf166 homolog		CN166_MOUSE	28	V–SVZ, RMS		Cytoskeleton

domain of GFP-tagged CLASP2 (Fig. 3C and D). The M domain of CLASP2 (CLASP2-M) was specifically detected in the GST-Girdin-CT pull-down sample, suggesting that CLASP2-M contains the Girdin-binding site (Fig. 3D). These results suggest that CLASP2 forms a complex with Girdin via CR2.

4. Discussion

In this study, we sought to uncover novel regulatory mechanisms for neuronal migration by screening for proteins that interact with Girdin, an actin-binding protein essential for neuronal migration from the V–SVZ to the OB in the postnatal mouse brain. Girdin is reported to be involved not only in neuronal migration but also in the motility of immature epithelial cells, such as cancer cells [25]. Since Girdin is involved in rearranging the actin cytoskeleton to control cell motility [17,25], we chose the molecules categorized as 'locomotion' for biological process and/or 'cytoskeleton' for cellular component, from the list of Girdin-interacting proteins in the Gene Ontology analysis (Table 1). These proteins included actin and actin cytoskeleton-binding proteins such as Drebrin, Ezrin, RCC2, ERC protein 2, and Spectrin. Drebrin plays an important role in dendritic spine formation and maintenance through actomyosin contraction [26,27]. Since Drebrin is involved in cell migration and expressed in new neurons in the V–SVZ and RMS [28,29], it is possible that Girdin interacts with Drebrin to control the actin rearrangement in neuronal migration.

In the list of Girdin-interacting proteins, we also identified some microtubule proteins and microtubule-associated proteins. DCLK1 and MARK1 are serine/threonine-protein kinases that regulate microtubule dynamics. DCLK1 is known to be involved in the Calcium signaling pathway controlling neuronal migration [30], and MARK1 is involved in neuronal migration by phosphorylating Dcx [31]. CLIP2, CLASP2, and MAP2 are microtubule-binding proteins. CLASP2 is reported to accumulate at the plus-end of microtubules and to control microtubule dynamics and coupling with the actin cytoskeleton [18,24]. We observed that CLASP2 co-localized with Girdin in new neurons in the V–SVZ, and found that Girdin preferentially interacts with the M domain of CLASP2. Taken together, these results suggest that an interaction between Girdin and CLASP2 is involved in neuronal migration. Girdin is also known as HkRP1 (Hook-related protein 1), a Dynamin-interacting protein [32]. The name HkRP1 derives from the fact that the NT domain of Girdin shares high sequence homology with the microtubule-binding domain of the Hook protein family, which links microtubules to the membrane compartment. Although there is little evidence that Girdin directly binds microtubules, a report using inhibitors suggest a functional association between microtubules and Girdin [32]. Our results suggest that an interaction between Girdin and certain microtubule-binding proteins, identified as Girdin-interacting proteins, regulates microtubule dynamics and neuronal migration.

In conclusion, using Girdin as a clue, this study identified novel proteins that might be involved in controlling the migration of new

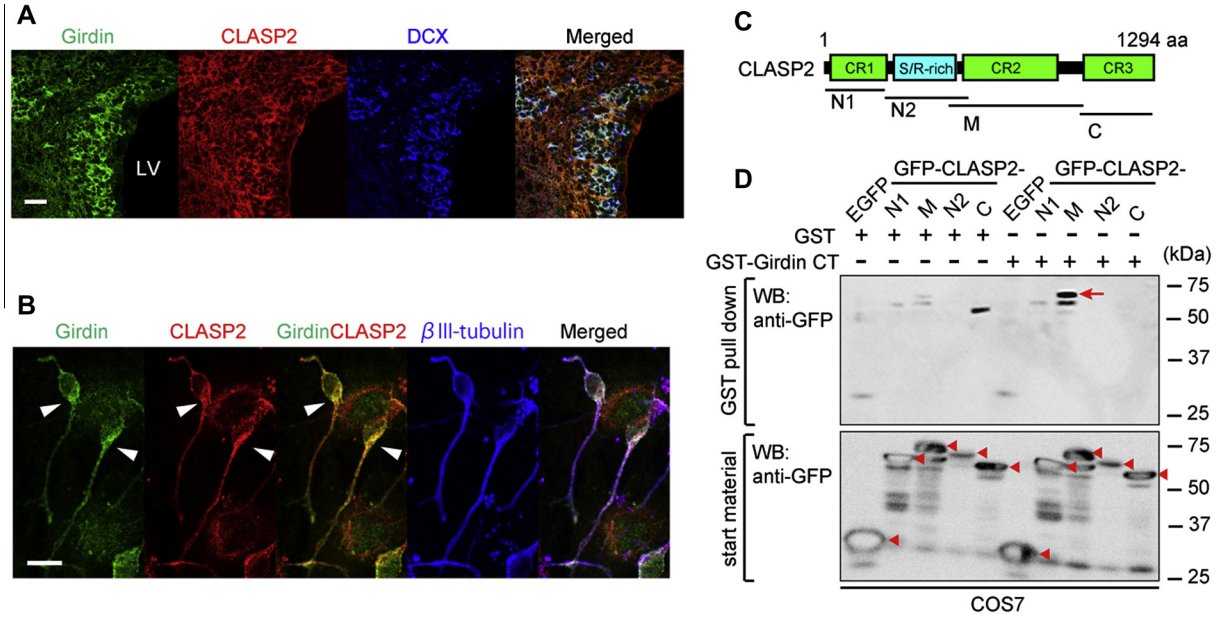

Fig. 3. Interaction between Girdin and CLASP2. (A) Expression of CLASP2 and Girdin in new neurons in the V–SVZ. Coronal sections from wild-type mouse brain were triple-stained with anti-Girdin (green), anti-CLASP2 (red), and anti-Dcx (blue) antibodies. Scale bar: 20 μm. (B) Co-localization of CLASP2 and Girdin in cultured new neurons from the V–SVZ. New neurons dissociated from the V–SVZ tissue of wild-type P1–4 mice were fixed after 2 days *in vitro*, followed by immunostaining with anti-Girdin (green), anti-CLASP2 (red), and anti-βIII tubulin (blue) antibodies. Arrowheads indicate the proximal leading process of new neurons. Scale bar: 10 μm. (C) Domain structures of CLASP2 and its fragments used in this study. CR, conserved region; S/R rich, serine/arginine-rich. (D) Pull-down analysis with the CT domain of Girdin fused to GST. (Top) The lysate of COS7 cells expressing EGFP or EGFP-CLASP2 fragments was incubated with GST or GST-Girdin-CT immobilized beads. The bound proteins were analyzed by immunoblotting with an anti-GFP antibody (GST-pull down). The M domain of CLASP2 was detected in the GST-Girdin-CT pull-down sample (red arrow). (Bottom) Total cell lysates (starting material) from COS7 cells expressing each domain of GFP-CLASP2 (red arrowheads) are shown.

neurons. The data suggest that Girdin and Girdin-interacting proteins control neuronal migration by regulating actin and/or microtubule dynamics. More detailed analyses of these candidates will increase our understanding of the mechanisms that control neuronal migration from the V–SVZ to the OB.

Author contributions

H.O., T.H., and K. Sawamoto designed the study; H.O., T.H., T.N., M.M., and J.I. performed the experiments and analyzed data; H.O., T.H., T.N., M.M., J.I., N.A., A.E., M.T., K.K., K. Sobue, and K. Sawamoto analyzed and interpreted the data; M.T., K.K., K. Sobue, and K. Sawamoto supervised the project; H.O., T.H., and K. Sawamoto wrote the manuscript; and all the authors discussed the results and agreed on the content of this manuscript.

Competing financial interests

The authors declare no competing financial interests.

Acknowledgments

This work was supported by the Funding Program for the Next Generation World-Leading Researchers (Japan Society for the Promotion of Science) and JSPS KAKENHI Grant Number 23700385. Liquid chromatography tandem mass spectrometry (LC–MS/MS) shotgun analyses were supported by a Grant-in-Aid for Scientific Research on Innovative Areas (Comprehensive Brain Science Network) from the Ministry of Education, Science, Sports and Culture of Japan.

References

[1] H.T. Ghashghaei, C. Lai, E.S. Anton, Neuronal migration in the adult brain: are we there yet?, Nat Rev. Neurosci. 8 (2007) 141–151.
[2] M.E. Hatten, New directions in neuronal migration, Science 297 (2002) 1660–1663.
[3] O. Marin, J.L. Rubenstein, Cell migration in the forebrain, Annu. Rev. Neurosci. 26 (2003) 441–483.
[4] M. Sawada, S. Huang, Y. Hirota, N. Kaneko, K. Sawamoto, Neuronal migration in the adult brain, in: T. Seki, K. Sawamoto, J.M. Parent, A. Alvarez-Buylla (Eds.), Neurogenesis in the Adult Brain I, Springer, Heidelberg, 2011, pp. 337–355.
[5] F. Doetsch, A. Alvarez-Buylla, Network of tangential pathways for neuronal migration in adult mammalian brain, Proc. Natl. Acad. Sci. USA 93 (1996) 14895–14900.
[6] A. Jankovski, C. Sotelo, Subventricular zone-olfactory bulb migratory pathway in the adult mouse: cellular composition and specificity as determined by heterochronic and heterotopic transplantation, J. Comp. Neurol. 371 (1996) 376–396.
[7] D.R. Kornack, P. Rakic, The generation, migration, and differentiation of olfactory neurons in the adult primate brain, Proc. Natl. Acad. Sci. USA 98 (2001) 4752–4757.
[8] C. Lois, A. Alvarez-Buylla, Long-distance neuronal migration in the adult mammalian brain, Science 264 (1994) 1145–1148.
[9] L. Petreanu, A. Alvarez-Buylla, Maturation and death of adult-born olfactory bulb granule neurons: role of olfaction, J. Neurosci. 22 (2002) 6106–6113.
[10] M.B. Luskin, Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone, Neuron 11 (1993) 173–189.
[11] G. Gheusi, H. Cremer, H. McLean, G. Chazal, J.D. Vincent, P.M. Lledo, Importance of newly generated neurons in the adult olfactory bulb for odor discrimination, Proc. Natl. Acad. Sci. USA 97 (2000) 1823–1828.
[12] M. Sakamoto, I. Imayoshi, T. Ohtsuka, M. Yamaguchi, K. Mori, R. Kageyama, Continuous neurogenesis in the adult forebrain is required for innate olfactory responses, Proc. Natl. Acad. Sci. USA 108 (2011) 8479–8484.
[13] M. Alonso, G. Lepousez, W. Sebastien, C. Bardy, M.M. Gabellec, N. Torquet, P.M. Lledo, Activation of adult-born neurons facilitates learning and memory, Nat. Neurosci. 15 (2012) 897–904.
[14] N. Kaneko, O. Marin, M. Koike, Y. Hirota, Y. Uchiyama, J.Y. Wu, Q. Lu, M. Tessier-Lavigne, A. Alvarez-Buylla, H. Okano, J.L. Rubenstein, K. Sawamoto, New neurons clear the path of astrocytic processes for their rapid migration in the adult brain, Neuron 67 (2010) 213–223.
[15] C. Lois, J.M. Garcia-Verdugo, A. Alvarez-Buylla, Chain migration of neuronal precursors, Science 271 (1996) 978–981.
[16] Y. Wang, N. Kaneko, N. Asai, A. Enomoto, M. Isotani-Sakakibara, T. Kato, M. Asai, Y. Murakumo, H. Ota, T. Hikita, T. Namba, K. Kuroda, K. Kaibuchi, G.L. Ming, H. Song, K. Sawamoto, M. Takahashi, Girdin is an intrinsic regulator of neuroblast chain migration in the rostral migratory stream of the postnatal brain, J. Neurosci. 31 (2011) 8109–8122.

[17] A. Enomoto, H. Murakami, N. Asai, N. Morone, T. Watanabe, K. Kawai, Y. Murakumo, J. Usukura, K. Kaibuchi, M. Takahashi, Akt/PKB regulates actin organization and cell motility via Girdin/APE, Dev. Cell 9 (2005) 389–402.
[18] T. Watanabe, J. Noritake, M. Kakeno, T. Matsui, T. Harada, S. Wang, N. Itoh, K. Sato, K. Matsuzawa, A. Iwamatsu, N. Galjart, K. Kaibuchi, Phosphorylation of CLASP2 by GSK-3beta regulates its interaction with IQGAP1, EB1 and microtubules, J. Cell Sci. 122 (2009) 2969–2979.
[19] A. Enomoto, N. Asai, T. Namba, Y. Wang, T. Kato, M. Tanaka, H. Tatsumi, S. Taya, D. Tsuboi, K. Kuroda, N. Kaneko, K. Sawamoto, R. Miyamoto, M. Jijiwa, Y. Murakumo, M. Sokabe, T. Seki, K. Kaibuchi, M. Takahashi, Roles of disrupted-in-schizophrenia 1-interacting protein girdin in postnatal development of the dentate gyrus, Neuron 63 (2009) 774–787.
[20] A. Keller, A.I. Nesvizhskii, E. Kolker, R. Aebersold, Empirical statistical model to estimate the accuracy of peptide identifications made by MS/MS and database search, Anal. Chem. 74 (2002) 5383–5392.
[21] A.I. Nesvizhskii, A. Keller, E. Kolker, R. Aebersold, A statistical model for identifying proteins by tandem mass spectrometry, Anal. Chem. 75 (2003) 4646–4658.
[22] M. Ashburner, C.A. Ball, J.A. Blake, D. Botstein, H. Butler, J.M. Cherry, A.P. Davis, K. Dolinski, S.S. Dwight, J.T. Eppig, M.A. Harris, D.P. Hill, L. Issel-Tarver, A. Kasarskis, S. Lewis, J.C. Matese, J.E. Richardson, M. Ringwald, G.M. Rubin, G. Sherlock, Gene ontology: tool for the unification of biology. The gene ontology consortium, Nat. Genet. 25 (2000) 25–29.
[23] K. Sawamoto, H. Wichterle, O. Gonzalez-Perez, J.A. Cholfin, M. Yamada, N. Spassky, N.S. Murcia, J.M. Garcia-Verdugo, O. Marin, J.L. Rubenstein, M. Tessier-Lavigne, H. Okano, A. Alvarez-Buylla, New neurons follow the flow of cerebrospinal fluid in the adult brain, Science 311 (2006) 629–632.
[24] A. Akhmanova, C.C. Hoogenraad, K. Drabek, T. Stepanova, B. Dortland, T. Verkerk, W. Vermeulen, B.M. Burgering, C.I. De Zeeuw, F. Grosveld, N. Galjart, Clasps are CLIP-115 and -170 associating proteins involved in the regional regulation of microtubule dynamics in motile fibroblasts, Cell 104 (2001) 923–935.
[25] P. Jiang, A. Enomoto, M. Jijiwa, T. Kato, T. Hasegawa, M. Ishida, T. Sato, N. Asai, Y. Murakumo, M. Takahashi, An actin-binding protein Girdin regulates the motility of breast cancer cells, Cancer Res. 68 (2008) 1310–1318.
[26] C. Aoki, N. Kojima, N. Sabaliauskas, L. Shah, T.H. Ahmed, J. Oakford, T. Ahmed, H. Yamazaki, K. Hanamura, T. Shirao, Drebrin a knockout eliminates the rapid form of homeostatic synaptic plasticity at excitatory synapses of intact adult cerebral cortex, J. Comp. Neurol. 517 (2009) 105–121.
[27] T. Mizui, N. Kojima, H. Yamazaki, M. Katayama, K. Hanamura, T. Shirao, Drebrin E is involved in the regulation of axonal growth through actin-myosin interactions, J. Neurochem. 109 (2009) 611–622.
[28] X.P. Dun, T. Bandeira de Lima, J. Allen, S. Geraldo, P. Gordon-Weeks, J.K. Chilton, Drebrin controls neuronal migration through the formation and alignment of the leading process, Mol. Cell. Neurosci. 49 (2012) 341–350.
[29] M. Song, N. Kojima, K. Hanamura, Y. Sekino, H.K. Inoue, M. Mikuni, T. Shirao, Expression of drebrin E in migrating neuroblasts in adult rat brain: coincidence between drebrin E disappearance from cell body and cessation of migration, Neuroscience 152 (2008) 670–682.
[30] P.T. Lin, J.G. Gleeson, J.C. Corbo, L. Flanagan, C.A. Walsh, DCAMKL1 encodes a protein kinase with homology to doublecortin that regulates microtubule polymerization, J. Neurosci. 20 (2000) 9152–9161.
[31] B.T. Schaar, K. Kinoshita, S.K. McConnell, Doublecortin microtubule affinity is regulated by a balance of kinase and phosphatase activity at the leading edge of migrating neurons, Neuron 41 (2004) 203–213.
[32] F. Simpson, S. Martin, T.M. Evans, M. Kerr, D.E. James, R.G. Parton, R.D. Teasdale, C. Wicking, A novel hook-related protein family and the characterization of hook-related protein 1, Traffic 6 (2005) 442–458.