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Arf6 guanine-nucleotide exchange factor cytohesin-2 regulates myelination in nerves

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ABSTRACT

In postnatal development of the peripheral nervous system (PNS), Schwann cells differentiate to insulate neuronal axons with myelin sheaths, increasing the nerve conduction velocity. To produce the mature myelin sheath with its multiple layers, Schwann cells undergo dynamic morphological changes. While extracellular molecules such as growth factors and cell adhesion ligands are known to regulate the myelination process, the intracellular molecular mechanism underlying myelination remains unclear. In this study, we have produced Schwann cell-specific conditional knockout mice for cytohesin-2, a guanine-nucleotide exchange factor (GEF) specifically activating Arf6. Arf6, a member of the Ras-like protein family, participates in various cellular functions including cell morphological changes. Cytohesin-2 knockout mice exhibit decreased Arf6 activity and reduced myelin thickness in the sciatic nerves, with decreased expression levels of myelin protein zero (MPZ), the major myelin marker protein. These results are consistent with those of experiments in which Schwann cell-neuronal cultures were treated with pan-cytohesin inhibitor SecinH3. On the other hand, the numbers of Ki67-positive cells in knockout mice and controls are comparable, indicating that cytohesin-2 does not have a positive effect on cell numbers. Thus, signaling through cytohesin-2 is required for myelination by Schwann cells, and cytohesin-2 is added to the list of molecules known to underlie PNS myelination.

1. Introduction

In the embryonic stage of the peripheral nervous system (PNS)'s development, Schwann lineage cells proliferate and migrate along neuronal axons to their final destinations. After birth, Schwann cells eventually wrap around individual axons to form the myelin sheath [1,2]. The myelin sheath is derived from the Schwann cell's morphologically differentiated plasma membrane. It not only insulates axons to increase their nerve conduction velocity but also protects them from various stresses such as physical damage. Myelin sheaths can grow to be more than one hundred times larger than the collective surface area of the premyelination Schwann cell plasma membranes. For this reason it is thought that the myelination process consists of dynamic cell morphological change. Myelination by Schwann cells is known to be promoted by growth factors such as neuregulin-1 and insulin-like growth factor 1, as well as by cell adhesion molecules such as integrin ligands, all of which are thought to be presented by peripheral neurons [1–6]. While these intercellular signals have already been identified, the intracellular molecular mechanism controlling the myelination process still remains to be established [5,6].

Arf6, an Arf member of the small GTPase family, is a unique, ubiquitous protein that plays key roles in both intracellular vesicular trafficking and cell morphological change [7–10]. Arf6's

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guanine-nucleotide state is controlled by two types of proteins, namely, guanine-nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). The former reaction is a key event, since GEFs define the intracellular compartment that activates Arf6 and/or its strength by integrating the upstream signals [7–10]. Cytohesin-2, the second of the four cytohesins, is one of these proteins and is widely expressed in tissues including nervous tissues [11,12]. All cytohesins share the same domain structure organization with a catalytic Sec7 domain and a phosphoinositide-binding pleckstrin homology (PH) domain [13].

In this study, we produced conditional cytohesin-2 knockout mice and investigated the role of cytohesin-2 in Schwann cell myelination in the PNS. Schwann cell-specific cytohesin-2 knockout mice exhibit decreased Arf6 activity in the nerves and reduced myelin thickness during development. This indicates that cytohesin-2 regulates myelination by Schwann cells, though it was not previously known that cytohesin-2 had any involvement in PNS myelination.

2. Materials and methods

2.1. Antibodies

The following antibodies were purchased: mouse monoclonal anti-cytohesin-2, mouse monoclonal anti-Arf1, mouse monoclonal anti-Arf6, and rabbit polyclonal Krox20 (also called Egr2; Schwann cell differentiation marker) from Santa Cruz Biotechnology (Santa Cruz, CA, USA); mouse monoclonal anti-myelin basic protein (MBP; myelin marker) from Covance (Princeton, NJ, USA); rabbit polyclonal anti-myelin protein zero (MPZ, also called P0; myelin marker) and mouse monoclonal anti-actin from MBL (Nagoya, Japan); mouse monoclonal anti-Ki67 antigen (cell proliferation marker) and rabbit polyclonal anti-active (cleaved) caspase 3 (apoptotic cell marker) from Cell Signaling Technology (Danvers, MA, USA); and peroxidase-conjugated secondary antibodies from GE Healthcare (Little Chalfont, UK) or Nacalai Tesque (Kyoto, Japan). Fluorescence-labeled secondary antibodies were obtained from Life Technologies (Carlsbad, CA, USA) or Wako (Osaka, Japan).

2.2. Production of conditional cytohesin-2 knockout mice and identification of MPZ promoter-controlled Cre recombinase transgenic mice

EuMMCR cytohesin-2 conditional knockout C57BL/6 mouse ES cells ($Cyth2^{tm1a(EUCOMM)Wtsi}$) were purchased from Eucomm (Munich, Bayern, Germany) and used by Unitech, Inc. (Chiba, Japan) to generate chimeric mice. To identify the *neo* gene-containing knockout cassette, offspring were genotyped by genomic PCR. For evaluation of the knockout cassette allele, the primers used were 5′-CTTCCTCGTGCTTTACGGTATC-3′ (sense) and 5′-ATCCAAAGTCTG AGATGCTAATGTC-3′ (antisense) for the knockout cassette's long arm position and 5′-TGACTACCTACGGGTAACAGTTTCT-3′ (sense) and 5′-CGTTAAAGTTGTTCTGCTTCATCA-3′ (antisense) for the knockout cassette's short arm position. In the former primer pair, the allele harboring knockout cassette displayed ~6600 base pairs (bps). In the later primer pair, the allele harboring knockout cassette displayed ~400 bps, enabling us to obtain four chimeric mice. Next, to remove the *neo* gene, offspring were mated with Unitech Flp recombinase transgenic mice. For evaluation of the *neo* gene deletion allele, the primers used were 5′-TCAGGA AATGTCTCTCAAATAAGAC-3′ (sense) and 5′-AAATCTCTGCTCCAAC TGTAGCTT-3′ (antisense) for the knockout cassette's short arm position and 5′-GAACAAGATGGATTGCACGCAGGTTCTCCG-3′ (sense) and 5′-GTAGCCAACGCTATGTCCTGATAG-3′ (antisense) for the *neo* gene. In the former primer pair, the knockout allele with the *neo* gene, the knockout allele without the *neo* gene (also called floxed allele), and the wild type allele displayed ~7300 bps, ~390 bps, and ~240 bps, respectively. This primer pair was used to determine the floxed allele in the following experiments. In the later primer pair, the allele with the *neo* gene displayed ~670 bps, enabling us to obtain five hemizygotic floxed knockout mice. Finally, to remove the *flp* gene, these mice were further mated with the wild type C57BL/6 mice (Sankyo, Inc., Tokyo, Japan). The primers used were 5′-TAGTTTGCAATTACAGTTCGAATCA-3′ (sense) and 5′-AGCCTTGTTGTACGATCTGACTAAG-3′ (antisense) for the *flp* gene. The allele with the *flp* gene displayed ~500 bps and the allele without the *flp* gene was selected.

To delete the *cytohesin-2* gene specifically in myelinating Schwann cells, cytohesin-2 conditional knockout mice were mated with MPZ promoter-controlled Cre recombinase transgenic mice (Stock No. 017927, Jackson Laboratory, Bar Harbor, ME, USA). The PCR primers used to identify the *cre* transgene were 5′-TTTGCCTGCATTACCGGTCGATGCAAC-3′ (sense) and 5′-GCGCGAGTTGATAGCTGGCTGGTG-3′ (antisense). The product was ~750 bps.

In all experiments, PCR amplification (using LA Taq polymerase or EX Taq polymerase; Takara Bio, Shiga, Japan) was performed in 30 cycles, each cycle consisting of denaturation at 94 °C for 1 min, annealing at 62.5–67.5 °C for 1 min, and extension at 72 °C for 1 or 5 min, depending on the primer pair's annealing temperature and the product length. Male mice were used for experiments if it was possible to distinguish their sex. Homozygous mice, as well as heterozygous mice, were fertile under standard breeding conditions.

2.3. Immunoblotting

Cells or tissues were lysed in lysis buffer A (50 mM HEPES-NaOH, pH 7.5, 20 mM $MgCl_2$, 150 mM NaCl, 1 mM dithiothreitol, 1 mM phenylmethane sulfonylfluoride, 1 μg/ml leupeptin, 1 mM EDTA, 1 mM Na_3VO_4, and 10 mM NaF) containing detergents (0.5% NP-40, 1%CHAPS, and 0.1%SDS). These detergents are important for protein isolation in the insides of myelin segments [14–17]. Unless otherwise indicated, all steps were performed at 4 °C. Equal amounts of the proteins in centrifuged cell supernatants were heat-denatured, subjected to polyacrylamide gels (Bio-Rad, Hercules, CA, USA), and blotted to PVDF membranes (Bio-Rad) using the Trans-Blot TurboTransfer System (Bio-Rad). Electrically transferred membranes were blocked with the Blocking One reagent (Nacalai Tesque) and immunoblotted using a primary antibody followed by a peroxidase-conjugated secondary antibody. The bound antibodies were detected using the chemiluminescence kit (Nacalai Tesque or Wako). Scanned protein bands were densitometrically analyzed using UN-SCAN-IT software (Silk Scientific, Orem, UT, USA).

2.4. Affinity precipitation assay to detect active Arf1 and Arf6

Cells or tissues were lysed in lysis buffer A containing detergents. Unless otherwise indicated, all steps were performed at 4 °C. The proteins in centrifuged cell supernatants were mixed for 1.5 h with *E. coli*-produced recombinant GST-tagged GGA3 proteins (20 μg of GGA3 proteins per 800 μg of total proteins from cell lysates) that had been preabsorbed with glutathion-resin (GE Healthcare), collected by centrifugation, and washed with lysis buffer A containing detergents [16]. GGA3 specifically binds to active, GTP-bound Arf1 or Arf6. The washed resins were heat-denatured and subjected to polyacrylamide gels for their respective immunoblotting experiments with anti-Arf1 or Arf6 antibodies.

2.5. Schwann cell-neuronal cultures

Dissociated explants were established from male or female C57BL/6 mice on embryonic day 12.5 [16]. In brief, dorsal root ganglia (DRGs) were collected and dissociated using 0.25% trypsin and trituration. The cells were dispersed and plated onto collagen type I-precoated coverslips (3×10^5 cells/22 mm-coverslip). The dissociated explants were maintained in MEM containing 10% FBS and 100 ng/ml NGF. Axonal processes and endogenous Schwann cells were allowed to grow and establish themselves for 5 days. Myelination was induced using 50 μg/ml ascorbic acid. The culture medium in the presence or absence of 10 μM SecinH3 was changed every 2 or 3 days and cultures were maintained for an additional 2 weeks.

2.6. Immunofluorescence study to detect myelin segments

Cells on collagen type I-precoated coverslips were air-dried, fixed in PBS containing 4% paraformaldehyde (Nacalai Tesque), treated with ice-cold methanol (Nacalai Tesque), and permeabilized with PBS containing 0.1% Triton X-100 (Nacalai Tesque). These steps are important for protein staining in the interiors of myelin segments [16]. Permeabilized cells were blocked with the Blocking One reagent and washed with PBS containing 0.1% Tween-20, then incubated first with primary antibodies and then with fluorescence-labeled secondary antibodies. Cells were then mounted for microscopic observation using the Vectashield with DAPI reagent (Vector Laboratories, Burlingame, CA, USA). The fluorescent images were captured using a DMI4000 or DMI4000B microscope system (Leica, Wetzlar, Germany) controlled by AF6000 software (Leica). Segments harboring more than 250 fluorescent maximum values were considered to be myelin segments. The number of these segments was counted in the 200 μm square field defined as the region of interest (ROI).

2.7. Immunohistochemistry

Tissues were perfused first with PBS and then with PBS containing 4% paraformaldehyde. They were postfixed with 4% paraformaldehyde, replaced with 20% sucrose, and embedded in the Tissue-Tek reagent (Sakura Finetechnical, Tokyo, Japan). Microtome sections on slide glasses were blocked with the Blocking One reagent, and incubated first with primary antibodies and then with fluorescence-labeled secondary antibodies. They were mounted using the Vectashield with DAPI reagent. Fuorescent images were captured using either a DM2500 microscope system (Leica) controlled by LAS software (Leica) or captured using a BX51 microscope system (Olympus, Tokyo, Japan) controlled by DP2-BSW software (Olympus).

2.8. Electron microscopic analysis

Sciatic nerves were fixed with 2% paraformaldehyde and 2% glutaraldehyde in 0.1% cacodylate buffer overnight. The tissues were postfixed with buffered 2% osmium tetroxide, dehydrated with an ethanol gradient, treated with acetone, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed with a H-7500 electron microscope system (Hitachi, Tokyo, Japan). About 30 micrographs at 2500–7000 folds' magnification were randomly taken, and the g-ratio of each axon was calculated using ImageJ software (http://imagej.nih.gov/ij/) by dividing perimeters of the axolemma with those of compact myelin.

2.9. Statistical analysis

Values shown represent the mean ± SD from separate experiments. One-way analysis of variance (ANOVA) was performed followed by a Fisher's protected least significant difference (PLSD) test as a post hoc comparison. A p value less than 0.05 was considered significant.

2.10. Experimental animal ethics

Genetically modified/unmodified mice were produced and maintained in accordance with a protocol approved by the Japanese National Research Institute for Child Health and Development Animal Care Committee.

3. Results

The aim of this study was to determine whether cytohesin-2 has the ability to control Schwann cell myelination. Myelination begins after birth, becomes very active during the first several weeks of life, and leads to the formation of mature myelin within two months [1,2]. In PNS sciatic nerves, expression profiles illustrated that cytohesin-2 protein is upregulated after birth and retains its elevated levels until adulthood (Fig. 1A and B). In contrast, control actin protein expression remained stable through all developmental stages after birth. These results suggest the involvement of cytohesin-2 in myelination. Therefore, we tried to produce conditional cytohesin-2 knockout mice that would be suitable for the Cre/loxP recombination system (Fig. 2A). Schwann cell-specific MPZ promoter-driven Cre recombinase transgenic mice were used for deletion of loxP-flanked genes [18]. Conditional deletion of cytohesin-2 was confirmed both by genomic PCR with specific primers using tail genomic DNAs and by immunoblotting with anti-cytohesin-2 antibody in sciatic nerves (Fig. 2B and C). Conditional knockout of cytohesin-2 did not affect expression levels of cytohesin-2 in other tissues such as brain and liver ones (Fig. S1).

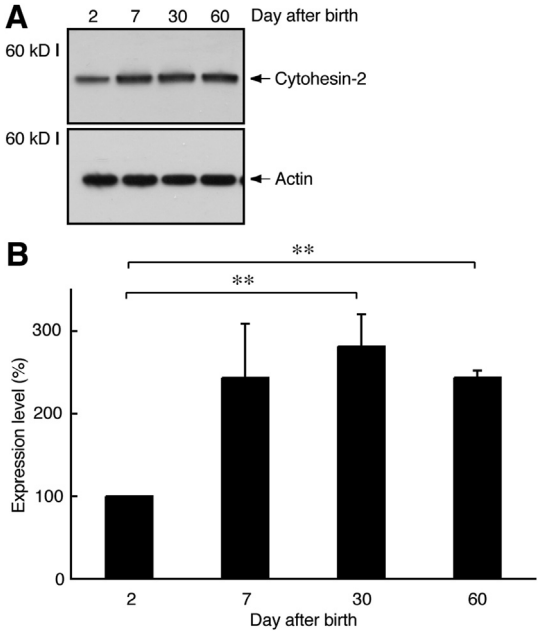

Fig. 1. Changes in expression levels of cytohesin-2 in sciatic nerves of the PNS. (A) Sciatic nerve extracts were immunoblotted with an antibody against cytohesin-2 or control actin. (B) The bands in the immunoblots were scanned and densitometrically analyzed (**, $p < 0.01$; n = 4 blots).

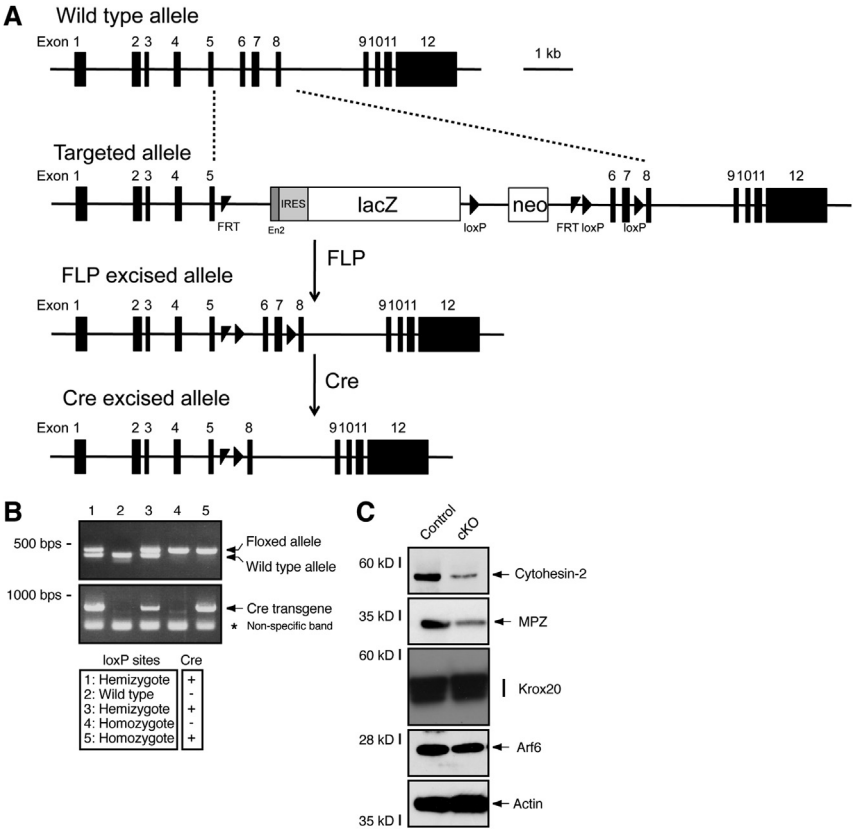

Fig. 2. Production of Schwann cell-specific cytohesin-2 conditional knockout mice. (A) Schematic diagram of our production of Schwann cell-specific cytohesin-2 (composed of 12 exons) conditional knockout mouse allele (1st allele map). By mating chimeric mice with Flp recombinase (FLP)-transgenic mice, the insert between short flippase recognition target (FRT) sequences (2nd allele map) was removed from the chimeric mouse genome harboring the conditional knockout potential cytohesin-2 exon unit, the En2 splice acceptor-IRES-LacZ sequence, and the neomycin-resistant gene, enabling us to obtain the floxed mice (3rd allele map). The insert between short loxP sequences was removed by mating these mice with Schwann cell-specific MPZ promoter-controlled Cre recombinase-transgenic mice (MPZ-Cre), producing Schwann cell-specific cytohesin-2 conditional knockout mice (4th allele map). (B) The representative data of genomic PCR using one littermate. The PCR primers used to identify the floxed allele were 5′-TCAG-GAAATGTCTCTCAAATAAGAC-3′ (sense) and 5′-AAATCTCTGCTCCAACTGTAGCTT-3′ (antisense). The floxed and wild type alleles displayed ~390 b and ~240 b, respectively (upper panel). The PCR primers used to identify the cre transgene were 5′-TTTGCCTGCATTACCGGTCGATGCAAC-3′ (sense) and 5′-GCGCGAGTTGATAGCTGGCTGGTG-3′ (antisense). The product was ~750 b (lower panel). In this case, the mouse corresponding to lane 5 is the conditional knockout (cKO) mouse, while that corresponding to lane 4 is the control mouse. (C) The tissue lysates from knockout mice or controls were immunoblotted with an antibody against cytohesin-2, MPZ, Krox20, Arf6, or the control actin.

Fig. 2B shows an example of the genotypes of littermate mice: lane 5 corresponds to the knockout mouse (conditional cytohesin-2 knockout alleles with the *cre* gene) and lane 4 to the littermate control mouse (conditional knockout alleles without the *cre* gene). In some experiments, mice with a single conditional cytohesin-2 knockout allele with the *cre* gene are often used as littermate controls. Since their cytohesin-2 expression was downregulated, these knockout mice exhibited decreased expression levels of MPZ, the major myelin marker protein [2]. In contrast, the expression levels of Krox20, a Schwann cell differentiation marker protein [2], were comparable in knockout mice and littermate controls, suggesting that cytohesin-2 affects regulation of the extent of myelination but not Schwann cell differentiation.

We next examined the relationship between knockout of cytohesin-2 and the activity of the effector Arf6. To measure the levels of GTP-bound Arf6 (active Arf6) in sciatic nerve tissue lysates, we performed the affinity precipitation method using a recombinant GGA3, which specifically binds to GTP-bound Arf6 and Arf1 [9,10]. Knockout mice exhibit decreased Arf6 activity, compared to littermate controls (Fig. 3A and B). The levels of GTP-bound Arf1, in contrast, were comparable in knockout mice and controls (Fig. 3C and D), illustrating that cytohesin-2 indeed has the ability to control the activity of Arf6 *in vivo*.

Since cytohesin-2 knockout mice exhibited decreased MPZ levels, we analyzed the myelin sheath ultrastructure in sciatic nerves. Electron microscopic analysis revealed decreased myelin thickness in the sciatic nerves from 5-day-old knockout mice exhibited decreased myelin thickness, compared to those from controls (Fig. 4A). This decreased myelin thickness in knockout mice is clearly evident from quantification of the average g-ratio, the numerical average ratio of the axon diameter to the diameter of the outermost myelinated fiber [1–6]. Thinner myelin sheaths yield larger average g-ratios (0.747 ± 0.0749 in knockout mice, compared to 0.679 ± 0.0589 in the controls; Fig. 4B). Similar results were observed in 60-day-old knockout mice (Fig. 4C), revealing that the reduction in myelin thickness persists throughout development (0.640 ± 0.0309 in knockout mice, compared to 0.603 ± 0.0372 in the controls; Fig. 4D).

We also established the long-term (approximately two-week-long) Schwann cell-neuronal explant cultures to confirm the ability of Schwann cells to form myelin sheaths. We first tried to establish cultures from knockout mice but failed to achieve this. Schwann cells did not attach to neurons well. Accordingly, we established Schwann cell-neuronal cultures from wild-type mice and treated them with pan-cytohesin inhibitor SecinH3 (10). SecinH3 inhibited myelination but did not obviously affect the cell

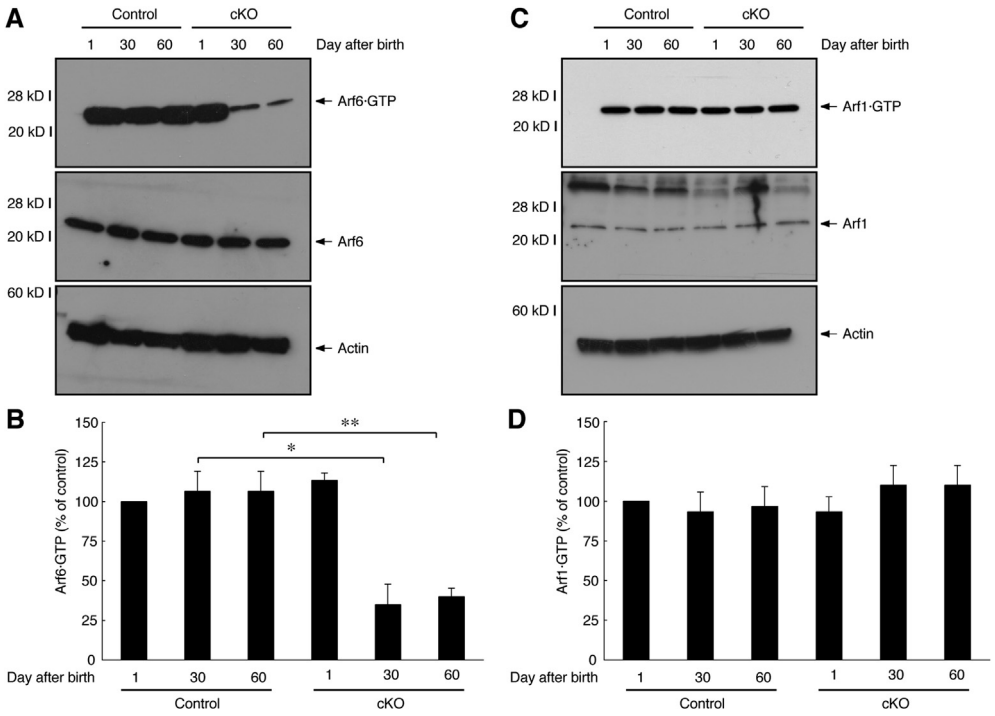

Fig. 3. Cytohesin-2 specifically activates Arf6 in mice. (A) 1-, 30-, or 60-day-old sciatic nerves from control or knockout mice were used for an affinity precipitation assay to detect GTP-bound Arf6. GTP-bound Arf6, total Arf6, and total actin are also shown. (B) The amounts of GTP-bound Arf6 are shown as relative values (*, $p < 0.05$, **, $p < 0.01$; n = 3 blots). (C) 1-, 30-, or 60-day-old sciatic nerves from control or knockout mice were used for an affinity precipitation assay to detect active, GTP-bound Arf1. GTP-bound Arf1, total Arf1, and total actin are also shown. (D) The amounts of GTP-bound Arf1 are shown as relative values (n = 3 blots).

number (Fig. S2). It is thus thought that cytohesin-2 has the ability to produce myelin sheaths, although it is also possible that certain Schwann cell cytohesins act collaboratively to produce them (16).

We also inquired whether knockout of cytohesin-2 affects cell numbers in sciatic nerves. Since the sciatic nerves are mainly composed of Schwann cells and nerve fibers without cell bodies, the number of marker-positive cells in these nerves is expected to be roughly equivalent to the number of marker-positive Schwann cells. The numbers of cell proliferation marker Ki67-positive cells [2] were comparable in knockout mice and controls (Fig. S3). In addition, the numbers of apoptotic cell marker active caspase 3-positive cells [2] were also comparable in both genotypes. These results reveal that knockout of cytohesin-2 does not have obvious effects on either proliferation or apoptosis in sciatic nerves.

4. Discussion

Myelination by Schwann cells requires continuous morphological changes, which are thought to be controlled through a complicated process by known and unknown signaling molecules [1–6]. It is assumed that some of these molecules are regulatory proteins switching signals on and off [5,6]. It is firmly established that small GTPases of the Rho family, such as Rac1, Cdc42, and RhoA, and their regulators, such as GEFs and GAPs, positively or negatively control cell morphogenesis. In the case of the Rho family GTPases, the regulators play key roles in integrating upstream signals and regulating Schwann cell myelination [3–6]. In fact, Rho GTPases cooperatively regulate multiple processes in Schwann cell myelination [3–6]. The Arf branch proteins of the small GTPase family participate not only in intracellular vesicle trafficking but also in cell morphogenesis; among them, Arf6 is best known to control cell morphogenesis [7–10]. In this study, we demonstrate that the Arf6-specific GEF cytohesin-2 [11,12] is required for Schwann cell myelination through experiments with our newly-produced Schwann cell-specific conditional knockout mice. Knockout of cytohesin-2 decreases the activity levels of Arf6, but not of Arf1, in nerves compared to controls. Cytohesin-2 knockout mice exhibit decreased myelin thickness during development, a finding consistent with the results from cultures. On the other hand, cytohesin-2 knockout does not obviously change the states of cells in nerves. Thus, cytohesin-2 is required for Schwann cell myelination. Although knockout of cytohesin-2 indeed decreases Arf6 activity, further studies will allow us to clarify how cytohesin-2, together with a cognate unidentified GAP, switches Arf6 on and off in a spatiotemporal manner in Schwann cells.

It has been established that Arf members, including Arf6, directly activate phosphatidylinositol-4-phosphate 5-kinases and phospholipase D isoenzyme, as do their effectors [7–10]. These enzymes generate phosphoinositides and phospholipids as products, triggering cell morphological changes. Therefore, these lipid-modifying enzymes may act downstream of cytohesin-2 and Arf6 in Schwann cells. Arf6 and other Arf members are also well known to bind to subunits of clathrin assembly protein complex 3 (AP3), where they control endocytosis [7–10], an important process causing cell morphological changes. Other adaptor proteins such as c-Jun N-terminal kinase (JNK)-interacting protein (JIP) 3 and JIP4 are also downstream targets of Arf6 [19]. JIP3 or JIP4 forms a complex with kinesin or dynactin to control microtube-dependent processes and regulate vesicle trafficking and cell morphogenesis. Also, Arf6 indirectly acts upstream of Rac1. Rac1 activation through Arf6 signaling is mediated by a possible interaction of cytohesin-2 with engulfment and cell motility (ELMO) 1 complex [20]. Arf6 effectors are involved in basic cellular functions such as lipid metabolism and controlling intracellular communication between signaling machines or much larger possible signalsomes. Therefore, they may coordinately act downstream of cytohesin-2 and Arf6 in this signaling cascade.

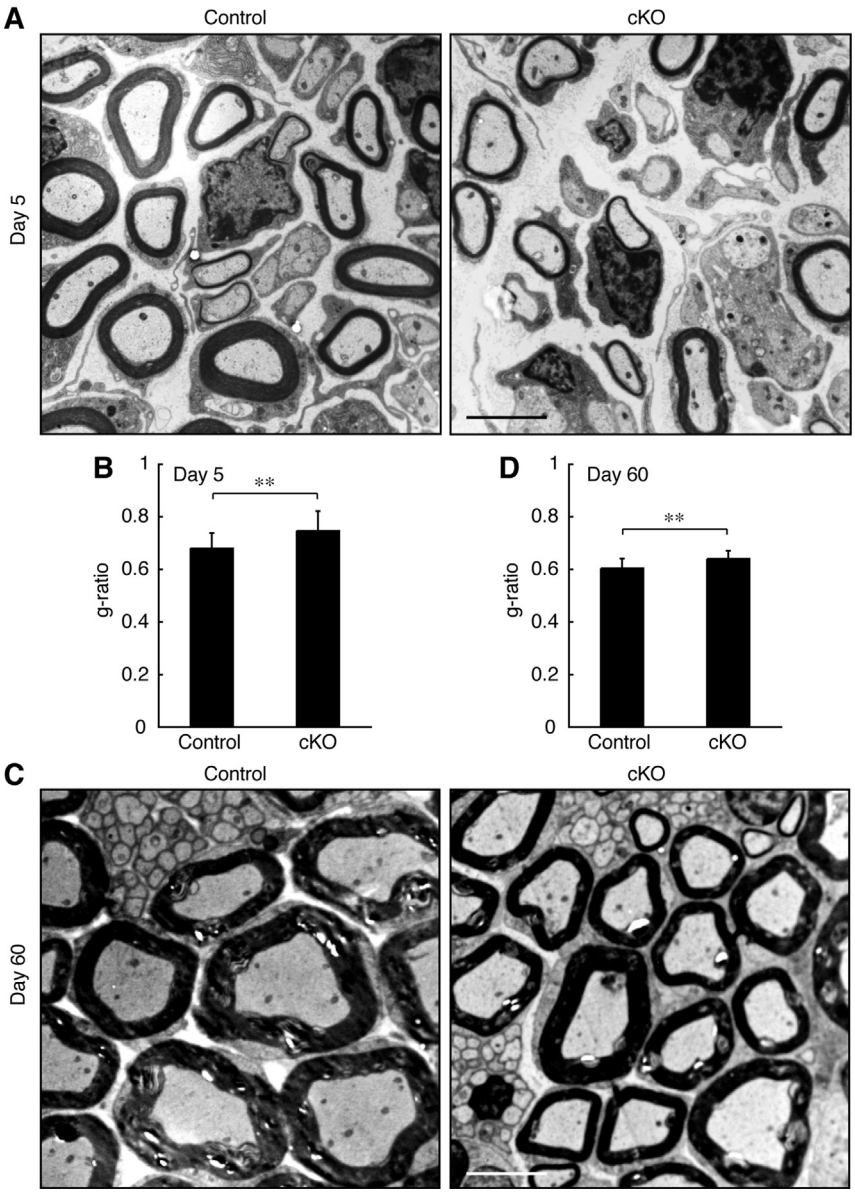

Fig. 4. Cytohesin-2 knockout mice decrease myelin thickness. Electron micrographs of the control or knockout mouse sciatic nerve cross section on postnatal day 5 (A; scale bar: 2 μm) and 60 (C; scale bar: 4 μm) are shown. (B, D) Average g-ratios of axon diameters to outer myelinated axon diameters in control and knockout mice are shown (**, $p < 0.01$; n = 50 nerves and 40 nerves on postnatal days 5 and 60, respectively).

Here, we show that the Arf6-specific GEF cytohesin-2 regulates myelination by Schwann cells *in vivo*. We previously characterized cytohesin-1, the first of the four cytohesins, as the regulator of Schwann cell myelination, mainly involved in the initiation of myelination [16]. Expression levels of cytohesin-1 are high in the early postnatal developmental stages but decrease during development [16]. It is thus conceivable that cytohesin-1 and cytohesin-2 both promote Arf6 activity during the initiation of myelination, and that cytohesin-2 may subsequently come to have a more central role in Arf6 activation. Further analyses using double conditional knockout mice for cytohesin-1 and cytohesin-2 may be needed to resolve this hypothesis. Additional studies will allow us to understand the detailed mechanism by which cytohesin-2 regulates myelination. Such studies may aid in developing a paradigm for remyelination and nerve regeneration.

Conflict of interest

None.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.03.113.

Transparency document

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