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DISC1 regulates synaptic vesicle transport via a lithium-sensitive pathway

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Abstract

Disrupted-In-Schizophrenia 1 (DISC1) is a susceptibility gene for major mental illnesses, including bipolar disorder and schizophrenia. Although the roles of DISC1 in nervous system development and functions are increasingly recognized, pathophysiological mechanisms underlying a range of neuropsychiatric symptoms caused by DISC1 mutations remain unclear. Here we show that DISC1 enhances synaptic vesicle transport along microtubules. Knocking down DISC1 expression results in attenuated vesicle transport in primary cortical neurons. Likewise, expressing the dominant-negative, breakpoint mutant version of DISC1 causes defective vesicle transport, by disrupting the assembly between the kinesin-1 adaptor FEZ1 and the cargo protein synaptotagmin-1 (Syt-1). In addition, lithium, a mood-stabilizing agent used for the treatment of bipolar disorder, can restore the assembly of FEZ1 and Syt-1, and normalizes the defective transport caused by the dominant-negative DISC1. Thus, this study addresses a new role of DISC1 in organelle transport in neurons, and suggests that this cellular pathway could be therapeutically targeted for the treatment against neuropsychiatric diseases.

Keywords

DISC1; FEZ1; lithium; bipolar disorder; vesicle transport; motor–cargo assembly

Introduction

Disrupted-in-schizophrenia 1 (DISC1) was originally identified as a gene located at the breakpoint of a balanced chromosomal translocation (1;11) (q42.1;q14.3) that cosegregates with schizophrenia, bipolar disorder, and major depression in a large Scottish pedigree (Millar et al., 2000). Although a series of recent studies delineated the roles of DISC1 in nervous system development and functions, including neurite outgrowth (Miyoshi et al., 2003; Ozeki et al., 2003), neuronal migration (Kamiya et al., 2005), synapse maintenance in matured neurons (Hayashi-Takagi et al., 2010), cAMP signaling (Millar et al., 2005), and

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neurogenesis (Duan et al., 2007; Mao et al., 2009; Ishizuka et al., 2011), it remains unknown how deficits of DISC1 underlie the pathology for neuropsychiatric symptoms, and which biological process can be targeted for most effective therapeutic intervention. This is in part due to multi-faceted functions of DISC1, as suggested by multiple DISC1 binding proteins with a diverse array of biological functions, and also due to the lack of comprehensive animal models that could reliably mimic the loss of each of these DISC1 functions.

Previous studies showed that DISC1 interacts with motor proteins, such as kinesin-1, its adaptor FEZ1, and components of dyneins in neurons, demonstrating the roles of DISC1 in critical aspects of neuronal development, including neurite outgrowth (Miyoshi et al., 2003; Ozeki et al., 2003; Taya et al., 2007) and neuronal migration (Kamiya et al., 2005). In addition, recent studies showed that FEZ1 binds and activates the kinesin-1 motor (Blasius et al., 2007), and that Unc-76, a *Drosophila* homolog of FEZ1, serves as a linker between kinesin-1 and the synaptic vesicle membrane protein Synaptotagmin-1 (Syt-1) and regulates synaptic vesicle transport in axons (Toda et al., 2008). These data suggest that DISC1 is a component of the kinesin-1-dependent motor machinery that plays a role in organelle transport.

In this study, we demonstrate a role of DISC1 in neuronal transport, either by knocking down DISC1 expression or by expressing the dominant-negative, breakpoint mutant version of DISC1 in primary cortical neurons. In addition, we address a new role of lithium in restoring vesicle transport that was attenuated by the dominant-negative DISC1 expression. These results revealed a key cellular process that is regulated by DISC1 and provides insight into the strategy we could consider as potential therapy for major mental illnesses.

Materials and Methods

Primary cortical neuron culture

The procedure for primary neuron preparation was approved by the Institutional Animal Care and Use Committee of Beckman Research Institute of City of Hope (Duarte, CA). Pregnant mice were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) cocktail during embryo isolation. Mouse cortical neurons were prepared from E13.5 embryos of timed-pregnant C57BL6/J mice by mechanical trituration through a Pasteur pipette, seeded on poly-D-lysine-coated glass cover-slips, and maintained in Neurobasal media (Invitrogen) supplemented with HEPES, sodium pyruvate, glucose, N-acetyl cysteine, B27, SPITE, oleic acid, linoleic acid, penicillin, and streptomycin. Cells were transfected at 5DIV using Lipofectamine 2000 (Invitrogen), as described (Hayashi-Takagi et al., 2010). GSK3 inhibitors used were: TDZD-8 (4-Benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione, Sigma; 5 μ M), SB216763 (3-(2,4-Dichlorophenyl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione, Cayman; 50nM), and BIO (6-bromoindirubin-3'-oxime, Calbiochem; 10nM). shRNA against mouse GSK3 (Open Biosystems) targets the following sequences: #1; 5'- CGGAGAGCCCAATGTTTCATAT -3' or #2; 5'- CCACAGAACCTCTTGTGGAT -3'

Time-lapse video microscopy

Mouse cortical neurons prepared from E13.5 embryos were cultured on a poly-D-lysine-coated 35-mm glass-bottom dish (MatTek), and transfected at 5DIV with expression plasmids encoding DISC1 shRNA (Kamiya et al., 2005) and VAMP2-GFP (pSUPER-shRNA_pCMV-VAMP2-GFP). Two days after transfection, time-lapse images were acquired every 3 sec (frame interval 3 sec) for 10–20 min, using Zeiss Observer system equipped with a temperature-controlled unit, a gas-chamber (5% CO₂, 37°C), a cooled charge-coupled device camera, and a 100x objective lens (NA=1.3). VAMP2-GFP-positive

puncta were individually tracked for every frame, and their average transport speeds (total distance traveled by each puncta/[3 sec x frame numbers]) were calculated by Image Pro Plus software (Media Cybernetics, Inc.).

Immunocytochemistry

Cells were fixed with 4% paraformaldehyde/PBS, and immunostained by standard indirect immunofluorescence method, using rabbit anti-GFP (1:1000, Molecular Probes) or rabbit anti-HA (1:1000, Abcam) followed by appropriate secondary antibodies conjugated with Alexa 488 or Alexa 546 (1:500, Molecular Probes).

Co-immunoprecipitation and Western blot

COS7 cells transfected with expression constructs were processed for immunoprecipitation (IP), as described (Toda et al., 2008). Rabbit anti-HA (Abcam) was used as an IP antibody, and the immunoprecipitates were analyzed by SDS-PAGE followed by Western blots using the following antibodies: anti-myc (1:250), anti-HA (1:1000), and anti-DISC1 (1:100) (gift of Dr. Joseph Gogos).

Results

DISC1 knockdown causes attenuated vesicle transport

To investigate the role of DISC1 in vesicle transport, we prepared primary cortical neurons from E13.5 mouse embryos, and knocked down DISC1 expression at day 5 in culture (5 days *in vitro* [DIV]), using two independent short-hairpin RNAs (shRNA) that could effectively silence DISC1 expression in neurons (Kamiya et al., 2005; Hayashi-Takagi et al., 2010). To visualize neuronal membrane vesicles, a synaptic vesicle membrane protein VAMP2 (vesicle-associated membrane protein 2) fused with GFP was included in the expression construct (Tomoda et al., 2004). At 7DIV, DISC1 knockdown resulted in aberrant distribution of VAMP2-GFP markers along neuronal processes (Fig. 1A). We observed a significant increase in the number of large VAMP2-positive puncta ($>1\mu\text{m}$ in diameter) in DISC1-knockdown neurons, suggesting that the vesicles tend to accumulate along the processes without being transported in the absence of DISC1. Co-transfection of DISC1 transgene restored the localization of VAMP2, supporting the role of DISC1 in normal vesicle distribution. We also addressed the integrity of microtubular structure and cell viability in DISC1-knockdown neurons, using EGFP-Tubulin and DAPI nuclear staining as markers. DISC1-knockdown neurons showed contiguous appearance of microtubules and had a nuclear morphology equivalent to that of control cells (Supplemental Fig. 1), suggesting that the defective transport is unlikely to be caused by aberrant microtubule formation or decreased cell viability. To directly address the effect of DISC1 knockdown on vesicle transport, VAMP2-GFP-positive puncta were imaged by time-lapse video microscopy (Fig. 1B, Supplemental Movie S1, S2). In neurons that express control shRNA, ~45% of the VAMP2-positive vesicles were mobile (faster than $0.1\mu\text{m/s}$), and their average speed was $0.54\pm0.06\mu\text{m/s}$ ($n=43$). In contrast, fewer than 10% of the vesicles were mobile in DISC1 knockdown neurons, and moved at significantly slower speeds (average $0.09\pm0.02\mu\text{m/s}$, $n=55$). Thus, DISC1 plays a critical role in vesicle transport.

DISC1 supports the assembly of the kinesin-1 adaptor and the synaptic vesicle cargo

Previous studies in *Drosophila* showed the role of Unc-76/FEZ1 in kinesin-1-mediated synaptic vesicle transport (Gindhart et al., 2003), and demonstrated that Unc-76 serves as a linker between kinesin-1 and synaptotagmin-1 (Syt-1), a synaptic vesicle membrane protein (Toda et al., 2008). A recent report confirmed that FEZ1 interacts with kinesin-1 in mammals (Blasius et al., 2007). To begin to address the molecular underpinning of DISC1-

mediated vesicle transport, we tested whether DISC1 can be included in the motor machinery complex responsible for synaptic vesicle transport (Fig. 2A). FEZ1 bound weakly to Syt-1 and this association was significantly enhanced by DISC1, suggesting a potential role of DISC1 in the assembly of the kinesin motor and the synaptic vesicle cargo.

DISC1 protein may be truncated as a result of the chromosomal translocation (1;11, q42.1;q14.3) (Millar et al., 2000), resulting in the generation of a dominant-negative DISC1 species. Our previous studies showed that this breakpoint mutant version of DISC1 titrates out the full-length DISC1 and impedes its normal function in neuronal migration, implying a dominant-negative mechanism associated with the DISC1 breakpoint mutation (Kamiya et al., 2005). Therefore, we next examined the effect of this breakpoint mutant of DISC1 on the assembly between FEZ1 and Syt-1 (Fig. 2B). The mutant DISC1 inhibited the FEZ1–Syt-1 binding in a dose-dependent manner, suggesting that DISC1 normally regulates the motor–cargo assembly.

Defective synaptic vesicle transport caused by the DISC1 mutation is normalized by lithium

Given that the DISC1 breakpoint mutation disrupted the FEZ1–Syt-1 interaction (Fig. 2B), we examined its effect on vesicle transport in primary cortical neurons. Transfection of the DISC1 breakpoint mutant resulted in a marked increase in the number of large VAMP2-positive vesicles ($\varphi > 1\mu\text{m}$) along the processes (Fig. 3A), and resulted in attenuated vesicle transport as revealed by time-lapse video microscopy (average puncta speed, $0.11 \pm 0.04\mu\text{m/s}$, $n=53$; Fig. 3B, Supplemental Movie S3), displaying a phenotype similar to that obtained with DISC1 knockdown (Fig. 1).

A recent report shows that the attenuated activity of DISC1 results in an increased glycogen synthase kinase 3 (GSK3) activity in neural precursor cells, thereby reducing the rate of neurogenesis, and that inhibiting the GSK3 activity rescues the neurogenic activity (Mao et al., 2009). By contrast, in post-mitotic neurons, increased GSK3 activity results in the phosphorylation of kinesin motor components, and negatively regulates kinesin-dependent transport (Morfini et al., 2002). Therefore, we tested whether the defective vesicle transport caused by the DISC1 breakpoint mutation can be rescued by inhibiting the GSK3 activity. Among the GSK3 inhibitors tested, lithium significantly reduced the numbers of large VAMP2-positive puncta along the processes in neurons transfected with the DISC1 breakpoint mutant (Fig. 3A), and upregulated vesicle transport (average puncta speed, $0.45 \pm 0.08\mu\text{m/s}$, $n=54$; Fig. 3B, Supplemental Movie S4), whereas additional GSK3 inhibitor compounds, TDZD-8, SB216763, and BIO, did not affect the accumulation of VAMP2-positive punctate structures (data not shown). To confirm the effect of GSK3 inhibition on vesicle transport, we knocked down GSK3 in neurons transfected with the DISC1 breakpoint mutant. The efficacy of GSK3 knockdown was confirmed in mouse embryonic fibroblasts infected with lentiviruses encoding two independent shRNAs against GSK3 (Supplemental Fig. 2). GSK3 knockdown did not significantly rescue the accumulation of VAMP2-positive puncta (Fig. 3A), suggesting that the rescuing activity of lithium is mediated through a pathway other than GSK3 inhibition.

To address the mechanism underlying the normalized VAMP2 distribution by lithium, we tested the effect of lithium on the FEZ1–Syt-1 interaction. Although the DISC1 breakpoint mutant downregulated the FEZ1–Syt-1 interaction, lithium treatment upregulated the FEZ1–Syt-1 interaction in a dose-dependent manner (Fig. 3C). The data raised the possibility that the effect of lithium may be mediated through the regulation of motor–cargo assembly. It remains to be tested whether lithium upregulates the motor–cargo assembly *in vivo*, using brain extracts from DISC1 dysfunction model mice after lithium administration.

Discussion

A number of studies reported the roles of DISC1 in key developmental steps, including neurite outgrowth (Miyoshi et al., 2003; Ozeki et al., 2003), neuronal migration (Kamiya et al., 2005), synapse maintenance (Hayashi-Takagi et al., 2010), cAMP signaling (Millar et al., 2005), and neurogenesis (Duan et al., 2007; Mao et al., 2009). Collectively, these studies strongly argue for the roles of DISC1 during critical neurodevelopmental periods, and early DISC1 dysfunction appears to manifest as a range of psychiatric symptoms later in life. Questions remain, however, as to which cell biological processes can be targeted for therapeutic intervention against neuropsychiatric symptoms once they manifested. One approach to address this question could be to identify aberrant cell biological pathways caused by DISC1 mutations and test whether a known compound used for the treatment of neuropsychiatric diseases can normalize those pathways.

We identified a new role of DISC1 in vesicle transport, in which DISC1 serves as a component of the transport machinery containing FEZ1, and appears to stabilize the formation of motor–cargo complex. In addition, we showed that DISC1 dysfunction causes a reduction in the transport speed of synaptic vesicles in neurons, and lithium can normalize the defective transport through a mechanism that does not involve GSK3 regulation. DISC1 may serve as a physical linker that enhances the motor-cargo affinity, or it could also be post-translationally modified by upstream signals and subsequently influence vesicle transport, as has been shown for other motor adaptor proteins, such as JIP1 and FEZ1 (Verhey et al., 2001; Blasius et al., 2007; Toda et al., 2008). Our current study is in line with a recent report, demonstrating that DISC1 polymorphism (Cys607Phe) affects mitochondrial transport in neurons (Atkin et al., 2011). It remains to be studied whether this or additional polymorphisms found in DISC1 could affect the transport of synaptic vesicles, and also whether DISC1 regulates the transport of additional cargoes in neurons. It should be noted that DISC1 interacts not only with kinesin motor machinery but also with dynein motor complex (Kamiya et al., 2005), which may regulate vesicle transport as well. Thus, further study is necessary to reveal detailed information on the components of DISC1-containing vesicle transport machinery.

Lithium is a mood-stabilizing agent that has been used over half a century for the treatment of bipolar disorder. It is notable that a series of recent genetic association and linkage studies have implicated DISC1 in bipolar disorder (Macgregor et al., 2004; Maeda et al., 2006; Carter 2007, Perlis et al., 2008). Multiple mechanisms have been proposed to explain lithium's action toward mood-stabilizing activity, including GSK3 inhibition (Klein and Melton, 1996), suppression of inositol metabolism (Berridge et al., 1989), activation of Akt through disruption of the β -arrestin–Akt–PP2A–GSK3 complex (Beaulieu et al., 2008), and neuroprotection against glutamate excitotoxicity, in part through activation of the cell survival signaling pathway or induction of neuroprotective proteins (Chuang, 2005). We do not know if any of these biochemical pathways can fully explain the cell biological effect of lithium on restoring vesicle transport attenuated by DISC1 dysfunction. Although GSK3 negatively regulates kinesin-1-mediated transport by phosphorylating kinesin light chain, a component of kinesin-1 (Morfini et al., 2002), GSK3 inhibition is unlikely to mediate the lithium's effect observed in our current study, because neither GSK3-inhibiting compounds nor GSK3 knockdown displayed the same effect as lithium. Inositol is a key component of phosphoinositides that are indispensable for many cellular signaling events, including intracellular membrane trafficking (Michell et al., 2006). It remains to be studied whether DISC1 dysfunction alters the inositol metabolism and if lithium can normalize vesicle transport by suppressing inositol metabolism in neurons. Akt is markedly activated by lithium (Beaulieu et al., 2008), and Akt-mediated phosphorylation of huntingtin at Ser 421 promotes anterograde vesicle transport in neurons (Colin et al., 2008; Zala et al., 2008).

Therefore, the effect of lithium on restoring the defective transport of synaptic vesicles may be mediated by Akt activation. It is possible that DISC1 normally promotes vesicle transport by translating the upstream Akt signaling activity to enhance the assembly of motor machinery. In this case, Akt activity may dynamically control the amount of DISC1 or other transport components included in the vesicle transport machinery. Alternatively, DISC1 may regulate Akt activity, thereby influencing vesicle transport. However, the levels of Akt activity reported for two models of DISC1 dysfunction appear contradictory (Hashimoto et al. 2006; Kim et al., 2009), with the former reporting a reduced level of Akt activity in DISC1 knockdown cortical neurons, whereas the latter reporting an increase in Akt activity in DISC1 knockdown hippocampal neurons. Therefore, further investigation is required to determine whether lithium restores defective vesicle transport via Akt pathway. Because uninterrupted transport of synaptic components is vital for neuronal function and viability (Guzik and Goldstein, 2004), the role of lithium in compensating for defective transport may contribute to the neuroprotective action elicited by lithium (Chuang, 2005).

Recent microarray studies suggest a link between lithium treatment and genes involved in multiple neuronal pathways, including synaptic vesicle trafficking, G-protein signaling, and metabolism (Fatemi et al., 2009; Gong et al., 2008; Liou et al., 2008; McMahon et al., 2006). Among these genes down-regulated by chronic lithium administration, *Vapa* gene (vesicle-associated membrane protein [VAMP]-associated proteins A) is associated with bipolar disorder (Lohoff et al., 2008). VAPA protein interacts with VAMP1 and VAMP2 (Weir et al., 1998), and is required for vesicular neurotransmission (Skehel et al., 1995). Because overexpression of VAPA impedes the endoplasmic reticulum-to-Golgi transport (Prosser et al., 2008), lithium-mediated suppression of *Vapa* may explain the lithium-induced upregulation of vesicle transport observed in our study.

Conclusions

In summary, we identified a new role of DISC1 in vesicle transport, which may be relevant to the pathophysiology of neuropsychiatric diseases, such as bipolar disorder. Further mechanistic studies using additional pharmacologic agents, combined with behavioral studies using animal models of DISC1 dysfunction, are expected to provide additional insights into the pathophysiological pathway that can be targeted for the treatment of major mental illnesses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- > DISC1 dysfunction causes defective vesicle transport in neurons.
- > DISC1 regulates synaptic vesicle transport by promoting the motor-cargo assembly.
- > Lithium can restore defective transport caused by DISC1 dysfunction.

Figure 1. DISC1 knockdown causes attenuated vesicle transport

(A) Primary cortical neurons were transfected with plasmids encoding a vesicle marker VAMP2-GFP, together with murine DISC1 shRNA (#1 or #2) or control shRNA (Ct), and stained with anti-GFP (green). For rescue experiments, cells were co-transfected with a plasmid encoding human DISC1 (red). Scale bar, 20µm. Numbers of VAMP2-positive puncta (>1µm in diameter) per 50µm neurite (mean±SEM) were plotted (graph). *Statistical significance ($p<0.01$, Student's t -test)

(B) Cortical neurons transfected with plasmids encoding a vesicle marker VAMP2-GFP, together with either control (Ct RNAi) or DISC1 shRNA#1 (mD1 RNAi), were observed by time-lapse video microscopy (frame interval, 3 sec). Arrowheads indicate the same puncta over the time sequence. Scale bars, 10µm (Ct RNAi), 20µm (mD1 RNAi).

Graph: % of VAMP2-positive puncta that move faster than 0.1µm/s were scored for each condition (upper). Average speed of VAMP2-positive puncta was measured for each condition (lower). *Statistical significance ($p<0.01$, Student's t -test)

Figure 2. DISC1 supports the assembly of FEZ1 and Syt-1

(A) COS7 cells were transfected with the indicated expression plasmids (full-length mouse DISC1, mouse FEZ1, and mouse Synaptotagmin-1 [Syt-1]) and processed for immunoprecipitation (IP) with anti-HA or the non-immune, control IgG, followed by Western blots (WB) using the indicated antibodies (anti-myc, HA).

(B) COS7 cells were transfected with the indicated expression plasmids (mouse DISC1 full-length [FL] or the breakpoint mutant [TR] (amino acids 1-594), mouse FEZ1, and mouse Synaptotagmin-1 [Syt-1]) ([−]=0μg, [+]=1μg, [++]=2μg) and processed for immunoprecipitation (IP) followed by Western blots as in (A).

Figure 3. Defective vesicle transport caused by the DISC1 breakpoint mutation is normalized by lithium

(A) Primary cortical neurons were co-transfected with plasmids encoding VAMP2-GFP, the murine DISC1 breakpoint mutant (DISC1/TR) or control (Ct), or with GSK3 shRNA at 5DIV, and processed for immunocytochemistry with anti-GFP at 7DIV. For drug treatment, 2mM LiCl was included in the culture media for the last 48 h of culture period. Scale bar, 20μm. Numbers of VAMP2-positive puncta (>1μm in diameter) per 50μm neurite (mean ±SEM) were plotted (graph). *Statistical significance ($p < 0.01$, Student's *t*-test)

(B) Cortical neurons co-transfected with plasmids encoding VAMP2-GFP and the DISC1 breakpoint mutant (DISC1/TR) were treated with or without 2mM LiCl for 48 h, and observed by time-lapse video microscopy (frame interval, 3 sec). Arrowheads indicate the same puncta over the time sequence.

(C) COS7 cells were transfected with the indicated expression plasmids (mouse DISC1 full-length [FL] or the breakpoint mutant [TR] (amino acids 1-594), mouse FEZ1, and mouse Synaptotagmin-1 [Syt-1]), incubated in the media containing the indicated concentration of LiCl for 48 h, and processed for immunoprecipitation (IP) with anti-HA, followed by Western blots (WB) using the indicated antibodies (anti-myc, HA, DISC1).