Published in final edited form as:

Biochem Biophys Res Commun. 2010 October 1; 400(4): 631–637. doi:10.1016/j.bbrc.2010.08.117.

Migration defects by DISC1 knockdown in C57BL/6, 129X1/SvJ, and ICR strains via *in utero* gene transfer and virus-mediated RNAi

Ken-ichiro Kubo^{a,#}, Kenji Tomita^{a,#}, Asuka Uto^a, Keisuke Kuroda^b, Saurav Seshadri^c, Jared S. Cohen^c, Kozo Kaibuchi^b, Atsushi Kamiya^{c,*}, and Kazunori Nakajima^{a,**}

^aDepartment of Anatomy, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo, Japan

^bDepartment of Cell Pharmacology, Graduate School of Medicine, Nagoya University, Showa, Nagoya, Japan

^cDepartment of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Abstract

Disrupted-in-Schizophrenia 1 (DISC1) is a promising genetic risk factor for major mental disorders. Many groups repeatedly reported a role for DISC1 in brain development in various strains of mice and rats by using RNA interference (RNAi) approach. Nonetheless, due to the complexity of its molecular disposition, such as many splice variants and a spontaneous deletion in a coding exon of the *DISC1* gene in some mouse strains, there have been debates on the interpretation on these published data. Thus, in this study, we address this question by DISC1 knockdown via short-hairpin RNAs (shRNAs) against several distinct target sequences with more than one delivery methodologies into several mouse strains, including C57BL/6, ICR, and 129X1/SvJ. Here, we show that DISC1 knockdown by *in utero* electroporation of shRNA against exons 2, 6, and 10 consistently results in neuronal migration defects in the developing cerebral cortex, which are successfully rescued by co-expression of full-length DISC1. Furthermore, lentivirus-mediated shRNA also led to migration defects, which is consistent with two other methodologies already published, such as plasmid-mediated and retrovirus-mediated ones. The previous study by Song's group also reported that, in the adult hippocampus, the phenotype elicited by DISC1 knockdown with shRNA targeting exon 2 was consistently seen in both C57BL/6 and 129S6 mice. Taken together, we propose that some of DISC1 isoforms that are feasible to be knocked down by shRNAs to exon 2, 6, and 10 of the *DISC1* gene play a key role for neuronal migration commonly in various mouse strains and rats.

Keywords

DISC1; *In utero* electroporation; Neuronal migration; Brain development

© 2010 Elsevier Inc. All rights reserved.

^{**}Corresponding author Department of Anatomy, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo, Japan Tel: +81-3-5363-3743, Fax: +81-3-5379-1977 kazunori@sc.itc.keio.ac.jp . ^{*}Corresponding author Department of Psychiatry and Behavioral sciences, Johns Hopkins University School of Medicine, 600N Wolfe St, Baltimore, MD 21208 USA Tel: +1-410-502-0060, Fax: +1-410-614-1792 akamiya1@jhmi.edu .

[#]These authors equally contributed to this work

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Disrupted-in-Schizophrenia-1 (DISC1), a promising genetic risk factor for major psychiatric disorders, has multiple roles in brain development [1-12]. Several independent groups have consistently demonstrated that DISC1 is important in regulating migration or coordinating the tempo of migration in a context-dependent manner, by using RNA interference (RNAi) [1, 4-6,8,10,12] (Table. 1). Nonetheless, because of the complexity of its molecular disposition, including many splice variants and a spontaneous deletion in a coding exon of the *DISC1* gene in some mouse strains [13-19], there have been many debates on the interpretation of these published data. This study is designed to address these questions systematically by focusing on radial neuronal migration in the developing cerebral cortex. Thus far, four independent groups have reported migration defects by knockdown of DISC1 in developing cerebral cortex (Table. 1). DISC1 short hairpin RNA (shRNA) targeted to sequences in exon 10 consistently leads to migration defects. Tsai and colleagues [10] reported this effect in Swiss Webster mice, an outbred strain, by plasmid-mediated shRNA via *in utero* electroporation. By using the same target sequences commonly conserved between rats and mice, Selkoe and Young-Pearse consistently found migration defects in Sprague Dawley rats [12]. The same intervention against exon 10 of *DISC1* also led to the similar defects in ICR mice, another outbred strain [4,5]. Song and colleagues used retrovirus-mediated shRNA targeting to exon 2 of *DISC1* and also reported the migration defect in the developing cerebral cortex in C57BL/6 [1]. Although these results from independent studies appear consistent, each study used different strains and species of animals and target sequences of RNAi, and different methods to deliver shRNA.

In this study, we compared the effects of three independent shRNAs to DISC1, including two already characterized, in plasmid-based *in utero* gene transfer. Importantly, the migration defects elicited by all these shRNAs were significantly rescued by co-expression with RNAi-resistant wild-type mouse DISC1, known as the full-length DISC1. The migration defects previously commonly reported in more than one outbred strain via DISC1 RNAi were reproduced in C57BL/6N. We also assessed how consistently we could elicit migration defects by DISC1 RNAi by a different delivering methodology, a lentivirus-mediated knockdown approach. Finally, we further characterized the time course of migration defects from prenatal to neonatal stages.

2. Materials and methods

2. 1. Plasmid constructs

Plasmids expressing shRNA were used for the suppression of DISC1 expression [20]. The effects of two shRNAs to DISC1 (RNAi-1 and -3) were well characterized in cell culture and *in utero* gene transfer by more than one group [4,5,7,9,12,21]. Another target sequence for RNAi to DISC1, previously characterized by retrovirus-mediated shRNA, was also used (RNAi-2) [1,6]. A scrambled target sequence without homology to any known messenger RNA was used to produce control RNAi (Con RNAi). Target sequences of these shRNAs to DISC1 were designed to distinct exons of DISC1 as follows.

RNAi-1 with mild suppression, 5'-CGGCTGAGCCAAGAGTTGG-3' in exon 6.

RNAi-2 with moderate suppression, 5'-CGGCTTCCAAGACACCTTC-3' in exon 2.

RNAi-3 with strong suppression, 5'-GGCAAACACTGTGAAGTGC-3' in exon 10.

Expression constructs of RNAi-resistant forms of mouse DISC1 with silent mutations in the target sequence of shRNAs (DISC1-R⁻¹, -R⁻², and -R⁻³ are resistant to DISC1 RNAi-1, -2, and -3, respectively) were tested for functional complementation in migration assays. Lentiviral shRNA vectors for knockdown of DISC1 (FUGW-D1) and control (FUGW-C1) were provided by Dr. Hongjun Song. Viral shRNA was produced by our published methods [21].

2. 2. In utero electroporation

C57BL/6NCr, ICR, and 129X1/SvJ pregnant mice (SLC) were used in this study. The day of vaginal plug occurrence was considered to be embryonic day 0 (E0). *In utero* electroporation was performed as described previously [4,5,22]. Briefly, DISC1 shRNAs (0.1-7.5 mg/ml) together with green fluorescent protein (GFP) expression vector (1 mg/ml) were injected into the lateral ventricle and electroporated into the ventricular zone at E15.0. RNAi-resistant forms of mouse DISC1 expression constructs on the pCAGGS1 vector [23] or pCAGGS1 vector (2.5 mg/ml) were co-electroporated to test functional complementation. All experiments were performed according to the institutional guidelines for animal experiments.

2. 3. Brain slice preparation and immunohistochemistry

Preparation of coronal slices of cerebral cortex and immunohistochemistry were performed as described previously [4,5,9,22,24,25]. Images from brain sections with self-inactivating lentiviral shRNA (pFUGW) [1,21] were captured after immunofluorescent staining with a rabbit polyclonal antibody against GFP (1:400; Molecular Probes). Slice images were acquired with confocal microscopes (FV300 or FV1000; Olympus, and LSM510; Carl Zeiss).

2. 4. In vivo bromodeoxyuridine (BrdU) labelling

BrdU (50 mg/kg body weight; Sigma) was injected intraperitoneally into pregnant mice 48 h after electroporation. Brain sections were stained with a rat monoclonal antibody against BrdU (1:1000, Abcam).

2. 5. Quantitative bin analysis of brain slices

To analyze the RNAi effect on cell positions, the cell positions of GFP-positive cells were quantified by bin analysis as previously described [5]. Statistical analyses were conducted with one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test. Student's t-test was used in comparing two sets of data. A value of $p < 0.05$ is considered statistically significant. Values were given as mean $\pm$ standard error of the mean.

2. 6. Cell culture and transfection

Cell culture and transfection were performed by our published protocols [4,5].

2. 7. Cell extraction and immunoblotting

Cell extraction and immunoblotting were performed by our published protocols [5,9]. The following primary antibodies were used for immunoblotting; mouse monoclonal antibodies against HA-tag (1:1000, Covance) and against β -tubulin (1:1000, Sigma-Aldrich). Quantitative densitometric measurement of Western blotting was performed by Image J.

3. Results

3. 1. Effect of RNAi targeting distinct exons of DISC1 on radial migration in the developing cerebral cortex

To validate the effects of RNAi to DISC1, we used three independent DISC1 shRNA constructs, DISC1 RNAi-1, -2, and -3, for targeting exon 6, 2, or 10, respectively (Fig. 1A). All DISC1 shRNAs were confirmed to suppress DISC1 protein expression, consistent with previous studies [1,4,5,7]. RNAi-1, -2, and -3 suppressed 64, 81, and 92% of mouse DISC1 protein expression, respectively, in HEK293T cells (Fig. 1B). RNAi-resistant forms of mouse DISC1 (mDISC1-R⁻¹, -2, and -3 are resistant to RNAi-1, -2, and -3, respectively) were also confirmed to be resistant to each shRNA by co-expression with the shRNA (data not shown).

We then tested the effects of DISC1 RNAi in the developing cerebral cortex in C57BL/6NCr mice by *in utero* gene transfer. Embryos were electroporated with DISC1 shRNAs expression construct together with GFP expression plasmid at embryonic day 15 (E15.0) and the effect of DISC1 knockdown on neuronal migration was evaluated by bin analysis of GFP-positive neurons at postnatal day 1 (P1.0). In brain sections with control shRNA, 59% of GFP-positive cells left the ventricular zone (VZ), subventricular zone (SVZ) and intermediate zone (IZ), and migrated into the upper cortical plate (CP) beneath the marginal zone (MZ) of the cortex (Fig. 1C). In contrast, in brain sections with DISC1 RNAi, a large number of GFP-positive neurons still remained in the midst of their migration in the CP, IZ, and VZ/SVZ (Fig. 1C). Of note, the effect of DISC1 RNAi on neuronal migration correlated with the level of DISC1 suppression, and was significantly restored by overexpression of RNAi-resistant forms of mouse DISC1 (DISC1-R⁻¹, -2, and -3), suggesting that disturbed migration by DISC1 RNAi did not originate from off-target effects (Fig. 1C and D). Migration defects by DISC1 RNAi-1 and -3 in ICR mice as well as migration defects by DISC1 RNAi-2 and -3 in 129X1/SvJ mice were also confirmed (Fig. S1). These phenotypes are unlikely to be non-specific effects of molecular disturbance by *in utero* electroporation, since overexpression of p27Kip1 (a cyclin-dependent kinase inhibitor [26]) at E15.0, which is known to mediate acceleration of migration [27], advanced migration by the same experimental paradigms (Fig. S2).

3. 2. Effect of DISC1 RNAi on neuronal migration via *in utero* lentivirus-mediated shRNA delivery

The effect of DISC1 knockdown was also been evaluated by an alternative approach, retrovirus-mediated shRNA delivery by *in utero* injection, which showed migration defect in the developing cerebral cortex [1,6]. To further confirm that migration defect is not a non-specific consequence of *in utero* electroporation, we tested another virus-mediated method, lentivirus-mediated RNAi. These RNAi constructs have the same target sequences as those used in the retrovirus-mediated RNAi, whose efficacies were well characterized [1,6]. Embryos were injected with lentivirus expressing DISC1 shRNA at E15.0 and the effect of DISC1 knockdown was evaluated at P1.0. As expected, knockdown of DISC1 by lentivirus expressing DISC1 shRNA also led to neuronal migration failure (Fig. 2A and B), suggesting that the migration defect was not caused non-specifically by the *in utero* electroporation. Of note, in contrast to the focal distribution of the transfected cells by *in utero* electroporation, lentivirus-infected cells showed wide distribution in developing brain, including the cerebral cortex and ganglionic eminences (GEs), main sources of GABAergic interneurons [28] (Fig. 2C).

3. 3. Altered cell positions caused by delayed migration during brain development

We next examined how migration defects by DISC1 knockdown would affect cell positions at different developmental stages. Brain slices with DISC1 RNAi-2 were used for time series analysis at E17.5, P1.0, P3.5, and P6.0 (Fig. 3A). The majority of GFP-positive cells with control RNAi were located at the top of the CP at P1.0 and were distributed in the middle of layers II-IV at P6.0, because the later-born GFP-negative cells had passed and piled up above the GFP-positive cells (Fig. 3B). In the brain sections with DISC1 RNAi-2, the majority of GFP-positive cells were still migrating throughout the CP and only a few had arrived beneath the MZ at P1.0 as shown in Fig. 1C. In contrast, a number of GFP-positive cells reached near the top of the CP at P3.5, suggesting that knockdown of DISC1 led to delayed migration, although a minor defect at the final step of radial migration near the MZ could also exist. By P6.0, most of the cells arrived near the MZ (Fig. 3).

3. 4. Effect of DISC1 RNAi on cell position at the postnatal stage via migration defect

We next compared the effect of DISC1 RNAi-1, -2, and -3 on cell positions at P6.0. Since the majority of the GFP-positive cells with shRNAs were distributed in the upper cortical layers

(II/III and IV) at P6.0 (Fig. S3A), the cell position within these layers was further quantified. We observed that the peak of distribution of GFP-positive cells with DISC1 shRNAs was shifted slightly to more superficial positions in layer II-IV, compared to those with Control RNAi (Fig. S3B and C). This phenotype was robust in cells with DISC1 RNAi-2, and cells with DISC1 RNAi-3, displayed a broad distribution in layer II-IV (Fig. S3). To confirm that the effect of DISC1 knockdown on cell position at P6.0 depended on the level of DISC1 suppression, we tested the effect of DISC1 RNAi-2 with varying doses: at concentrations of 1.0 mg/ml (low dose), 2.5 mg/ml (middle dose), and 7.5 mg/ml (high dose), and analyzed migration phenotypes at P6.0. In these experiments, we injected BrdU at E17.0, 2 days after electroporation, to label neurons born later than the electroporated neurons. As expected, the cells with control RNAi were mainly distributed deeper in the cortex than BrdU-labeled cells, reflecting the “inside-out” fashion of cortical layer formation (Fig. 4). Many cells with the low and middle dose of DISC1 RNAi-2 were found more superficially in the cortex, overlapping with the distribution of the BrdU-labeled cells more than control cells. On the other hand, a majority of cells with the high dose of DISC1 RNAi-2 was distributed in middle and deep positions in the cortex, similar to the result of DISC1 RNAi-3 (Fig. S3B), reflecting a defect in the radial migration toward the MZ. Taken together, these results suggest that cortical migrating neurons can eventually reach near the MZ with a delay when DISC1 is weakly or moderately suppressed, while strong suppression of DISC1 causes severe delay or arrest in radial migration. Consequently, at P6.0, weakly or moderately suppressed neurons are located more superficially than control cells, while strongly suppressed neurons are distributed in deep positions.

Discussion

There are three main findings in this study. First, we showed that suppression of DISC1 leads to radial neuronal migration defect in the developing cerebral cortex in C57BL/6Ncr mice, a common phenotype elicited by three independent shRNAs targeting distinct exons of *DISC1* by *in utero* electroporation. Our present data together with previous studies, including other groups, indicate that such migration defect elicited by DISC1 shRNA also occurs in ICR, Swiss Webster, and 129X1/SvJ mice as well as Sprague Dawley rats [4,5,8,10,12]. All RNAi effects were significantly normalized by overexpression of RNAi-resistant forms of mouse full-length DISC1, indicating that migration defects are truly associated with DISC1, but unlikely with off-target effects unrelated to DISC1 functions.

These conclusions in this study, however, may raise new important questions. In outbred strains, such as ICR and Swiss Webster mice, some but not all of them, carry a spontaneous 25-base pair deletion in a coding exon of *DISC1* (unpublished data), that was originally reported in 129 strains [13,14]. This mutation leads to generation of a stop codon by the frameshift of the non-triplet deletion and can theoretically deplete the known full-length DISC1 isoform resulting in about a 100 kDa protein [14]. Nonetheless, the patterns of DISC1 immunoreactivity between the 129 mice and C57BL/6 mice without this mutation are very similar [29]. Since this observation was confirmed by many investigators working on DISC1 with most of the antibodies available against DISC1, it is unlikely that this conclusion is just simply due to cross-reactivity of antibodies. How can we reconcile these data? One of the most reasonable interpretations at present is that there are unidentified functional isoforms of DISC1 even in the presence of the 25-base pair deletion. This idea is supported by the observation that a mutant DISC1 model originally utilized this 129-driven genetic deletion led only to impaired working memory [14], whereas many other DISC1 animal models, even those with single amino acid point mutations, display a greater range of abnormal behavior [30]. These data indicate that the 25-base pair deletion may not robustly affect overall DISC1 functions, although it is likely to delete the known full-length isoform. A recent paper indicating a similar migration defect of the adult dentate gyrus in both C57BL/6 and 129 mice by the knockdown of DISC1 via

retrovirus-mediated RNAi approach further supports this idea [6]. We believe that via taking systematic experiments and careful interpretation, the molecular disposition of DISC1 is, although still complicated, a key to elucidate its diverse and important functions in mental illnesses.

Second, we observed defective migration of the cells infected with lentivirus expressing DISC1 shRNA, which is consistent with the previous study with oncoretrovirus-mediated RNAi [1]. Since many cells are ubiquitously infected in several brain regions, including the GE, a major source of cortical interneurons [28], cells that did not originate from the pallial VZ/SVZ might be mixed with neurons from pallial VZ/SVZ in the CP. Thus, electroporation of shRNA-expression plasmids is a better approach, at least for addressing the developmental processes of specific cell populations in a distinct brain region.

Third, we also demonstrated that many cells with delayed migration by DISC1 knockdown seemed eventually to reach near the MZ at later time points. Thus, the timing of observation of cell positions is very important in examining the effect of DISC1 RNAi on neuronal migration. Nonetheless, conventional shRNA may not be temporally well controlled for the modulation of gene expression. DISC1 has multiple roles in various cellular events in brain development [1,2,4,5,7,8-12]. Thus, to address the molecular mechanisms precisely, it is important to segregate their functions in specific cellular processes. In this respect, the use of *in utero* electroporation with inducible and cell type specific gene targeting systems [31,32] would be expected for further exploration of DISC1-mediated molecular pathways and cell behaviors in brain development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Drs. Hongjun Song, Takeshi Kawauchi, and Jun-ichi Miyazaki for lentiviral RNAi constructs, p27Kip1 expression construct, and the CAG promoter, respectively. We thank members of Nakajima laboratory for valuable discussions and Ms. Yukiko Lema for organizing the manuscript. This work was supported by grants from Health Labour Sciences Research Grant (K.K.), Japan Society for the Promotion of Science (K.N.), Ministry of Education, Culture, Sports, and Science and Technology of Japan (K.K. and K.N.), the Takeda Science Foundation (K.N.), the Naito Foundation (K.N.), the Japan Brain Foundation (K.N.), the Promotion and Mutual Aid Corporation for Private Schools of Japan (K.N.), MH-091230 (A.K.), NARSAD (A.K.), and S-R (A.K.).

References

- [1]. Duan X, Chang JH, Ge S, et al. Disrupted-In-Schizophrenia 1 regulates integration of newly generated neurons in the adult brain. *Cell* 2007;130:1146–1158. [PubMed: 17825401]
- [2]. Enomoto A, Asai N, Namba T, et al. Roles of disrupted-in-schizophrenia 1-interacting protein girdin in postnatal development of the dentate gyrus. *Neuron* 2009;63:774–787. [PubMed: 19778507]
- [3]. Jaaro-Peled H, Hayashi-Takagi A, Seshadri S, et al. Neurodevelopmental mechanisms of schizophrenia: understanding disturbed postnatal brain maturation through neuregulin-1-ErbB4 and DISC1. *Trends Neurosci* 2009;32:485–495. [PubMed: 19712980]
- [4]. Kamiya A, Kubo K, Tomoda T, et al. A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. *Nat Cell Biol* 2005;7:1167–1178. [PubMed: 16299498]
- [5]. Kamiya A, Tan PL, Kubo K, et al. Recruitment of PCM1 to the centrosome by the cooperative action of DISC1 and BBS4: a candidate for psychiatric illnesses. *Arch Gen Psychiatry* 2008;65:996–1006. [PubMed: 18762586]
- [6]. Kim JY, Duan X, Liu CY, et al. DISC1 regulates new neuron development in the adult brain via modulation of AKT-mTOR signaling through KIAA1212. *Neuron* 2009;63:761–773. [PubMed: 19778506]

- [7]. Mao Y, Ge X, Frank CL, et al. Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3 β /beta-catenin signaling. *Cell* 2009;136:1017–1031. [PubMed: 19303846]
- [8]. Meyer KD, Morris JA. Disc1 regulates granule cell migration in the developing hippocampus. *Hum Mol Genet* 2009;18:3286–3297. [PubMed: 19502360]
- [9]. Niwa M, Kamiya A, Murai R, et al. Knockdown of DISC1 by in utero gene transfer disturbs postnatal dopaminergic maturation in the frontal cortex and leads to adult behavioral deficits. *Neuron* 2010;65:480–489. [PubMed: 20188653]
- [10]. Singh KK, Ge X, Mao Y, et al. Dixdc1 is a critical regulator of DISC1 and embryonic cortical development. *Neuron* 2010;67:33–48. [PubMed: 20624590]
- [11]. Taya S, Shinoda T, Tsuboi D, et al. DISC1 regulates the transport of the NUDEL/LIS1/14-3-3epsilon complex through kinesin-1. *J Neurosci* 2007;27:15–26. [PubMed: 17202468]
- [12]. Young-Pearse TL, Suth S, Luth ES, et al. Biochemical and Functional Interaction of Disrupted-in-Schizophrenia 1 and Amyloid Precursor Protein Regulates Neuronal Migration during Mammalian Cortical Development. *J Neurosci* 2010;30:10431–10440. [PubMed: 20685985]
- [13]. Clapcote SJ, Roder JC. Deletion polymorphism of Disc1 is common to all 129 mouse substrains: implications for gene-targeting studies of brain function. *Genetics* 2006;173:2407–2410. [PubMed: 16751659]
- [14]. Koike H, Arguello PA, Kvajo M, et al. Disc1 is mutated in the 129S6/SvEv strain and modulates working memory in mice. *Proc Natl Acad Sci U S A* 2006;103:3693–3697. [PubMed: 16484369]
- [15]. Kvajo M, McKellar H, Arguello PA, et al. A mutation in mouse Disc1 that models a schizophrenia risk allele leads to specific alterations in neuronal architecture and cognition. *Proc Natl Acad Sci U S A* 2008;105:7076–7081. [PubMed: 18458327]
- [16]. Ishizuka K, Paek M, Kamiya A, et al. A review of Disrupted-In-Schizophrenia-1 (DISC1): neurodevelopment, cognition, and mental conditions. *Biol Psychiatry* 2006;59:1189–1197. [PubMed: 16797264]
- [17]. Nakata K, Lipska BK, Hyde TM, et al. DISC1 splice variants are upregulated in schizophrenia and associated with risk polymorphisms. *Proc Natl Acad Sci U S A* 2009;106:15873–15878. [PubMed: 19805229]
- [18]. Hennah W, Thomson P, Peltonen L, et al. Genes and schizophrenia: beyond schizophrenia: the role of DISC1 in major mental illness. *Schizophr Bull* 2006;32:409–416. [PubMed: 16699061]
- [19]. Brandon NJ, Millar JK, Korth C, et al. Understanding the role of DISC1 in psychiatric disease and during normal development. *J Neurosci* 2009;29:12768–12775. [PubMed: 19828788]
- [20]. Brummelkamp TR, Bernards R, Agami R. A system for stable expression of short interfering RNAs in mammalian cells. *Science* 2002;296:550–553. [PubMed: 11910072]
- [21]. Hayashi-Takagi A, Takaki M, Graziane N, et al. Disrupted-in-Schizophrenia 1 (DISC1) regulates spines of the glutamate synapse via Rac1. *Nat Neurosci* 2010;13:327–332. [PubMed: 20139976]
- [22]. Tabata H, Nakajima K. Efficient in utero gene transfer system to the developing mouse brain using electroporation: visualization of neuronal migration in the developing cortex. *Neuroscience* 2001;103:865–872. [PubMed: 11301197]
- [23]. Niwa H, Yamamura K, Miyazaki J. Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene* 1991;108:193–199. [PubMed: 1660837]
- [24]. Nakajima K, Mikoshiba K, Miyata T, et al. Disruption of hippocampal development in vivo by CR-50 mAb against reelin. *Proc Natl Acad Sci U S A* 1997;94:8196–8201. [PubMed: 9223338]
- [25]. Tabata H, Nakajima K. Multipolar migration: the third mode of radial neuronal migration in the developing cerebral cortex. *J Neurosci* 2003;23:9996–10001. [PubMed: 14602813]
- [26]. Kawauchi T, Chihama K, Nabeshima Y, et al. Cdk5 phosphorylates and stabilizes p27kip1 contributing to actin organization and cortical neuronal migration. *Nat Cell Biol* 2006;8:17–26. [PubMed: 16341208]
- [27]. Nguyen L, Besson A, Heng JI, et al. p27kip1 independently promotes neuronal differentiation and migration in the cerebral cortex. *Genes Dev* 2006;20:1511–1524. [PubMed: 16705040]
- [28]. Marin O, Rubenstein JL. Cell migration in the forebrain. *Annu Rev Neurosci* 2003;26:441–483. [PubMed: 12626695]

- [29]. Ishizuka K, Chen J, Taya S, et al. Evidence that many of the DISC1 isoforms in C57BL/6J mice are also expressed in 129S6/SvEv mice. *Mol Psychiatry* 2007;12:897–899. [PubMed: 17895924]
- [30]. Clapcote SJ, Lipina TV, Millar JK, et al. Behavioral phenotypes of Disc1 missense mutations in mice. *Neuron* 2007;54:387–402. [PubMed: 17481393]
- [31]. Kamiya A. Animal models for schizophrenia via in utero gene transfer: understanding roles for genetic susceptibility factors in brain development. *Prog. Brain Res* 2009;179:9–15. [PubMed: 20302813]
- [32]. LoTurco J, Manent JB, Sidiqi F. New and improved tools for in utero electroporation studies of developing cerebral cortex. *Cereb Cortex* 2009;19(Suppl):i120–125. [PubMed: 19395528]

Fig. 1. Knockdown of DISC1 by RNAi targeting distinct exons leads to migration defects in the developing cerebral cortex

(A) A schematic representation of the mouse DISC1 exons targeted by shRNA constructs. DISC1 RNAi-1, -2, and -3 target exon 6, 2, and 10 of DISC1, respectively. (B) The graph represents densitometry analysis of western blotting after normalization by the expression level of β -tubulin. Con RNAi, control RNAi. (C) RNAi constructs and GFP expression vectors were electroporated into the ventricular zone (VZ) at E15.0 and analyzed at P1.0. 59% of GFP-positive cells terminated migration into the upper cortical plate (CP). By contrast, only 23, 12, and 4% of GFP-labeled cells reached in the most superficial region (Bins 9 and 10) with DISC1 RNAi-1, -2, and -3, respectively, of which migration defect was normalized by co-electroporation of RNAi-resistant wild-type mouse DISC1 (DISC1-R¹, -2, or -3). SVZ, subventricular zone. IZ, intermediate zone. Green, GFP. Magenta, propidium iodide (PI). Scale bar, 100 μ m. Error bars indicate SEM. (D) The effect of DISC1 shRNAs on migration correlates

with the suppression level of DISC1 expression. Overexpression of RNAi-resistant form of DISC1 rescued migration defect. Error bars indicate SEM. * $p < 0.01$.

Fig. 2. Effect of DISC1 RNAi on neuronal migration via *in utero* lentivirus-mediated RNAi
 (A) Lentivirus expressing shRNA and GFP were injected into the lateral ventricle at E15.0 and analyzed at P1.0. In the brains with Control RNAi (FUGW-C1), 21% of GFP-positive cells located into the superficial region of CP. By contrast, only 6 % of GFP-labeled cells reached the superficial region of CP in brain slices with DISC1 RNAi-D1 (FUGW-D1). Green, GFP. Blue, DAPI. Scale bar, 100 μ m. Error bars indicate SEM. (B) Silencing of DISC1 by lentiviral RNAi induces migration defect. Error bars indicate SEM. * $p < 0.05$. (C) The distribution of lentivirus-infected cells in forebrain at P1.0. GFP-positive cells were diffusely detected in forebrain, including cerebral cortex (CC) and ganglionic eminence (GE). Scale bar, 300 μ m.

Fig. 3. The changes of cell position provoked by knockdown of DISC1 depends on developmental stage

(A) Time-course analysis of cell position with knockdown of DISC1. The effect of DISC1 RNAi-2 on neuronal migration was analyzed at E17.5, P1.0, P3.5, and P6.0. In brains with Con RNAi, most of GFP-positive cells were located at the superficial positions in the CP after P1.0, whereas the majority of GFP-positive cells with DISC1 RNAi-2 were in the IZ at P1.0, and a number of these cells reached near the superficial positions of the CP by P3.5. WM, white matter. Scale bar, 100 μ m. Error bars indicate SEM. (B) High magnification images of brain sections with Con RNAi and DISC1 RNAi-2 at P3.5 and P6.0. Scale bar, 50 μ m.

Fig. 4. Effect of DISC1 RNAi on the cell position at the postnatal stage via migration defect
 (A) The effect of DISC1 RNAi-2 on the final cell position at P6.0 at different doses. 1.0 mg/ml (low dose), 2.5 mg/ml (middle dose), and 7.5 mg/ml (high dose) of DISC1 RNAi-2 was tested in the brains with BrdU-labeling at E17.0. Many cells with the low or middle dose of DISC1 RNAi-2 were found in the regions overlapping with many BrdU-positive cells, whereas cells with the high dose of DISC1 RNAi-2 distributed in the middle and deep positions in the cortex. Blue, BrdU-labeling cells. Scale bar, 100 μ m. Error bars indicate SEM. (B) Relative position of cells with the low and middle dose of DISC1 RNAi-2 was slightly higher, and lower with the high dose, compared with control RNAi. Error bars indicate SEM. * $p < 0.05$.

Table. 1
The effect of DISC1 Knockdown on neuronal migration

The role for DISC1 in neuronal migration have been examined in several mouse strains and rat, using RNA interference (RNAi), via *in utero* gene transfer and virus-mediated knockdown approach by *in vivo* injection. CC, cerebral cortex; DG, dentate gyrus.

Stage	Region	RNAi	Target exon	Species/Strain	Phenotype	References
Development	CC	Plasmid	10	Mouse/Swiss Webster	Disturbed migration	[10]
		Plasmid	10 and 6	Mouse/ICR	Disturbed migration	[4,5]
		Plasmid	10	Rat/Sprague Dawley	Disturbed migration	[12]
		Retrovirus	2	Mouse/C57BL/6	Disturbed migration	[1]
		Plasmid	2, 6, and 10	Mouse/C57BL/6, ICR, 129X1/SvJ	Disturbed migration	Current Study
		Lentivirus	2	Mouse/C57BL/6	Disturbed migration	Current Study
Adult	DG	Plasmid	2	Mouse/Swiss Webster	Disturbed migration	[8]
		Retrovirus	2	Mouse/C57BL/6	Overextended migration	[1]
		Retrovirus	2	Mouse/C57BL/6 and 129S6	Overextended migration	[6]