DISC1 regulates astrogenesis in the embryonic brain via modulation of RAS/MEK/ERK signaling through RASSF7

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ABSTRACT

Disrupted in schizophrenia 1 (DISC1) is known as a high susceptibility gene for schizophrenia. Recent studies have indicated that schizophrenia might be caused by glia defects and dysfunction. However, there is no direct evidence of a link between the schizophrenia gene DISC1 and gliogenesis defects. Thus, an investigation into the involvement of DISC1 (a ubiquitously expressed brain protein) in astrogenesis during the late stage of mouse embryonic brain development is warranted. Here, we show that suppression of DISC1 expression represses astrogenesis in vitro and in vivo, and that DISC1 overexpression substantially enhances the process. Furthermore, mouse and human DISC1 overexpression rescued the astrogenesis defects caused by DISC1 knockdown. Mechanistically, DISC1 activates the RAS/MEK/ERK signaling pathway via direct association with RASSF7. Also, the pERK complex undergoes nuclear translocation and influences the expression of genes related to astrogenesis. In summary, our results demonstrate that DISC1 regulates astrogenesis by modulating RAS/MEK/ERK signaling via RASSF7 and provide a framework for understanding how DISC1 dysfunction might lead to neuropsychiatric diseases.

KEY WORDS: DISC1, Astrogenesis, RAS/MEK/ERK signaling, MAP kinase, RASSF7, Cortical development

INTRODUCTION

Disrupted in schizophrenia 1 (DISC1) was originally identified in a chromosome translocation (1; 11) (q42.1; q14.3) in a Scottish family with a high susceptibility to schizophrenia and several other psychiatric disorders (Hayashi-Takagi et al., 2010; Millar et al., 2000). It is a crucial element of the microtubule-associated dynein motor complex and is important for maintaining normal microtubular dynamics (Kamiya et al., 2005). Induction of mutant forms of DISC1 in mouse brains led to the manifestation of schizophrenia-like behavioral phenotypes (Li et al., 2007; Pletnikov et al., 2008). Clapcote and colleagues generated two transgenic mouse lines with N-ethyl-N-nitrosourea-induced mutations of DISC1. The Q31L mutant mouse exhibited depressive-like behavior whereas the L100P mutant mouse showed schizophrenic-like behavior (Clapcote et al., 2007). Kuroda et al. showed that Disc1 mutant mice lacking exons 2 and 3 displayed abnormal behaviors with lower anxiety and higher impulsivity (Kuroda et al., 2011).

Previous studies have revealed that DISC1 is important in embryonic and adult brain development and is involved in the processes of neurogenesis, neuronal migration and axon formation. Kamiya et al. discovered that DISC1 depletion impairs neurite outgrowth (Kamiya et al., 2005). Enomoto et al. reported that DISC1 regulates axonal development by interacting with the protein girdin (CCDC88A; also known as KIAA1212) (Enomoto et al., 2009). Mao et al. provided evidence that DISC1 deficiency decreases the progenitor pool and results in premature neuronal differentiation by regulating GSK3β/β-catenin signaling. DISC1 dysfunction in the dentate gyrus of adult brain suppresses neural progenitor proliferation and causes hyperactive and depressive behaviors (Mao et al., 2009). Kim et al. showed that DISC1 modulates AKT/mTOR signaling, thereby regulating neuronal development (Kim et al., 2009). Most research into DISC1 has been focused on the effects on neurons, but few studies have investigated the role of DISC1 in astrocytes.

Astrocytes, the most abundant type of glial cells in the nervous system, impact the function of surrounding neurons in various ways (López-Hidalgo and Schummers, 2014). During forebrain development, neural stem cells generate neurons first, followed by astrocytes, and finally oligodendrocytes. This temporal sequence of neocortex development is tightly regulated by both intrinsic programs and extracellular signals (Brambilla et al., 2013). Astrocytes originate from progenitor cells in the subventricular zone (SVZ) during late embryonic development and reach a peak level in early postnatal life (Sauvageot, 2002). Glial progenitors, derived from common progenitors, can differentiate into astrocytes (Huse and Holland, 2010).

Astrocytes physically interact with neurons, offer metabolic support, buffer extracellular ions, and modulate information processing and signal transmission by releasing trophic factors and neurotransmitters (Annunziato et al., 2013; Barros, 2013; Pantazopoulos et al., 2010), and several depressive mouse models show defects in glial cell density (Molofsky et al., 2012). Therefore, we hypothesize that DISC1 dysfunction might affect astrogenesis, resulting in brain defects. Therefore, it is important to understand the molecular mechanism by which DISC1 regulates astrogenesis.

DISC1 is reported to act as a scaffold protein and interact with various partner proteins. In neurogenesis, KIAA1212 has been shown to associate with both C-terminal (CT) and middle domain...
(MD) domains of DISC1 when DISC1 regulates new neuron development in the adult brain. In addition, LIS1 (PAFAH1B1), NDE1, NDEL1 (Brandon et al., 2004; Burdick et al., 2008), PDE4B (Fatemi et al., 2008; Pickard et al., 2007) and DIXDC1 (Singh et al., 2010) have been previously reported to interact physically with DISC1. However, the protein involved in the function of DISC1 on astrogenesis remains elusive.

In this study, we focus on the regulation of astrogenesis by DISC1 during the late stages of embryonic brain development and the underlying molecular mechanisms. Using in utero electroporation and in vitro approaches with DISC1 shRNA or expression plasmids, we found that DISC1 regulates astrogenesis in the embryo brain: DISC1 depletion suppresses astrogenesis and DISC1 overexpression promotes the process. Moreover, human and mouse DISC1 overexpression rescued the astrogenesis defects caused by mouse DISC1 knockdown. A series of experiments demonstrated that DISC1 phosphorylates MEK and ERK downstream of RAS and then upregulates the expression of astrocyte-related genes. Furthermore, we identified RASSF7 as the molecule directly interacting with DISC1: interaction studies revealed that the CT domain of DISC1 was directly associated with the CT domain of RASSF7, a RAS association protein. In summary, these results indicate that DISC1 is required for astrogenesis in the embryonic brain and improve our understanding of the pathophysiology of brain diseases.

RESULTS

DISC1 expression in brain development

To test whether DISC1 regulates astrogenesis at the late stage of embryonic development, we first examined the correlation of DISC1 expression and astrogenesis in brain. Previous studies indicate that DISC1 is crucial to embryogenesis and organ development (Pickard et al., 2007; Singh et al., 2010). We analyzed DISC1 expression in different tissues and different stages of brain development by western blot analysis. The results showed that DISC1 expression was much higher in the brain than in other tissues (Fig. 1A). DISC1 expression increased from embryonic day (E) 12 to postnatal day (P) 0, and was maintained at a high level postnatally. ACSBG1 (related to astrocyte development) and GFAP (astrocyte marker) expression also increased during this period (E12–P0) (Fig. 1B; Fig. S1A–C). To determine DISC1 expression in cell culture, embryonic neural precursor cells (NPCs) were isolated and cultured in differentiation medium for 0, 1 and 3 days. Western blotting showed that the expression of ACSBG1 and GFAP increased along with the increasing expression of DISC1 (Fig. 1C). Immunostaining revealed colocalization of DISC1 and ACSBG1 and of DISC1 and GFAP in differentiated astrocytes (Fig. 1D, E). These results suggest that the initiation of astrogenesis might coincide with the expression of DISC1. In addition, in vivo immunostaining of brain slices at E16 showed that DISC1 was highly expressed and restricted to the ventricular zone (VZ)/SVZ (Fig. S1D). These data suggest that DISC1 might be broadly involved in brain development, including astrocyte differentiation.

DISC1 depletion represses astrogenesis in vitro and in vivo

To investigate further the regulation of astrogenesis by DISC1, a series of DISC1 knockdown experiments were performed in vitro and in vivo. First, four shRNAs were constructed into a lentiviral vector and the knockdown efficiencies were examined (Fig. S2A). The results showed that shRNA2 and shRNA3 had high knockdown efficiency. Subsequently, shRNA2, which had the highest knockdown efficiency, was used for the most of the knockdown experiments. We also confirmed that the DISC1 level decreased in vivo compared with the control after in utero electroporation (IUE) of DISC1 shRNA2 (Fig. S2B). Next, we studied the effect of DISC1 on astrocyte differentiation after E15 NPCs were infected with the DISC1 shRNA2 lentivirus. Western blotting and GFAP immunocytochemistry showed that DISC1 shRNA2 inhibited GFAP expression, and the number of NPCs that differentiated into astrocytes dramatically decreased without leukemia inhibitor factor (LIF) treatment (Fig. 2A, B; Fig. S2C). Because astrogenesis initiatives and continues during late embryonic development, control or DISC1 shRNA2 constructs were electroporated into the lateral ventricle of embryos at E16 in vivo. Brains were then harvested at P2, sectioned and stained.

Fig. 1. DISC1 expression in brain development. (A) Western blotting analysis of DISC1 protein in brain, muscle and four other tissues. Quantification of DISC1 expression levels is shown below (normalized to β-actin). Error bars indicate s.e.m. (n =3). (B) Western blot analysis of the level of DISC1, ACSBG1 and GFAP proteins in the brain cortex from E12 to P4. (C) Embryonic neural precursor cells (NPCs) were isolated and cultured in differentiation medium for 0, 1 and 3 days (D0, D1, D3). Western blot analysis of the expression of DISC1, ACSBG1 and GFAP is shown. (D) Colocalization of DISC1 and ACSBG1 in differentiated astrocytes. (E) Colocalization of DISC1 and GFAP in differentiated astrocytes. Scale bars: 10 μm. See also Fig. S1.
for markers of different glial subtypes at different stages of astrogenesis. According to previous studies, common progenitor cells give rise to glial progenitor cells, astrocyte progenitor cells and astrocytes during astrogenesis. Here, we used GLAST (SLC1A3), FGFR3 and GFAP to label glial progenitor cells, astrocyte progenitor cells and astrocytes, respectively (Li et al., 2012; Molofsky et al., 2012; Pringle, 2003). The immunostaining results revealed that the numbers of GLAST-positive glial progenitor cells, FGFR3-labeled astrocyte progenitor cells and GFAP-labeled astrocytes were reduced by 42.4%, 67.8% and 57.5%, respectively, in response to DISC1 knockdown (Fig. 2C-E), and a concomitant decrease of astrocytes by DISC1 knockdown could be seen in postnatal mouse brains after electroporation at P0, at which time neurogenesis is almost finished (Fig. S2D).

Moreover, rare morphologically mature astrocytes were observed in brains electroporated with DISC1 shRNA2 at the later stage (Fig. 2F). To exclude off-target effects, another plasmid (DISC1 shRNA3) was used to examine astrocyte differentiation in vitro and in vivo. Consistent with shRNA2, shRNA3 knockdown decreased GFAP expression and the number of GFAP-GFP-positive cells (Fig. S2E,F). Collectively, these data suggest that DISC1 is necessary for astrocyte differentiation.

**DISC1 overexpression enhances astrogenesis**

The astrogenesis defect caused by DISC1 knockdown prompted us to perform reciprocal DISC1 gain-of-function experiments. To analyze further the role of DISC1 during astrogenesis, we created a mouse DISC1 (mDISC1) overexpression plasmid to increase efficiently the DISC1 expression level in vitro (Fig. 3A; Fig. S3A); immunostaining of Flag showed that the Flag-mDISC1 had high overexpression efficiency (Fig. S3B). Moreover, the results obtained from the immunocytochemistry and western blot analyses showed that mDISC1 significantly promoted astrogenesis and increased the expression of GFAP in vitro (Fig. 3A,B). The amount of GFAP-positive cells was increased by 55.2% (Fig. 3B). Additionally, mDISC1 overexpression in vivo by electroporation increased the number of GLAST-positive radial progenitor cells, FGFR3-positive astrocyte precursor cells and GFAP-positive...
astrocytes (Fig. 3C-E). Moreover, the morphology of astrocytes electroporated with the DISC1 overexpression plasmid was typically more mature and complex in vivo (Fig. 3F). Interestingly, expression of GFAP and ACSBG1 was decreased and the number of GFAP+ astrocytes was reduced when the mutant DISC1 (DISC1-L100P) was overexpressed (Fig. S3C,D). Taken together, these results indicate that DISC1 overexpression facilitates astrogenesis both in vitro and in vivo.

**DISC1 overexpression rescues astrogenesis defects caused by DISC1 depletion**

To further examine DISC1 as a key regulator in astrogenesis, we generated a human DISC1 (hDISC1) overexpression plasmid and performed rescue experiments. First, the DISC1 expression level in the experiments was examined (Fig. S4A). Then, western blotting and immunostaining were used to examine the effect of hDISC1 on astrogenesis. The results showed a higher level of GFAP expression and an increased number of GFAP+ astrocytes when hDISC1 was overexpressed (Fig. 4A,B; Fig. S4B). After IUE in vivo, we observed that DISC1 overexpression and the astrocyte number were increased when hDISC1 and DISC1 shRNA were co-expressed (Fig. 4C,D; Fig. S4C). Additionally, mDISC1 could also rescue the astrocyte differentiation defect caused by DISC1 knockdown (Fig. S4D). These data suggest that mDISC1 or hDISC1 overexpression could restore the astrogenesis defects caused by mDISC1 knockdown.

**DISC1 regulates astrogenesis by modulating pMEK and pERK levels**

Multiple signaling pathways are reportedly involved in gliogenesis, including the JAK/STAT, MEK/ERK, and NOTCH pathways (Bonni et al., 1997; Li et al., 2012; Zhou et al., 2010). Li et al. found that the RAS/MEK/ERK signaling pathway plays a crucial role in activating the expression of astrocyte-specific genes (Li et al., 2012). Furthermore, the activation of RAS, MEK and ERK are three major nodes in the pathway. To test whether DISC1 regulates astrogenesis via this pathway, we checked the level of phosphorylated and total ERK after treatment with LIF. The data showed that LIF activated ERK (Fig. S5A). Western blot analysis showed that the levels of phosphorylated (p)MEK and pERK decreased due to DISC1 knockdown and increased due to DISC1 overexpression (Fig. 5A,B; Fig. S5B). We further observed that overexpression of ERK could rescue the deficits of astrocytes caused by DISC knockdown in vivo (Fig. 5C). The active form of ERK (pERK) is reported to function following translocation into the nucleus (Clark et al., 2004). Thus, we detected the location of pERK treated with LIF for different time intervals after DISC1 was knocked down or overexpressed. The results showed that pERK was primarily located in the cytoplasm in the absence of treatment, transported to the nucleus after 10 min of short-term treatment with LIF, and increasingly re-localized to the cytoplasm after 2 h (Fig. S5C). Collectively, these results indicate that DISC1 regulates astrogenesis by modulation of pMEK and pERK levels.
DISC1 regulates the RAS/MEK/ERK signaling pathway by direct interaction with RASSF7

Co-immunoprecipitation (co-IP) analysis showed that DISC1 does not directly interact with ERK, MEK or c-RAF (RAF1) (Fig. S5D). Therefore, we hypothesize that DISC1 may directly interact with an upstream component of the RAS pathway. Human RASSF7, a member of the RAS-association domain family, was previously reported as a potential binding partner of human DISC1 based on yeast two-hybrid screens (Morris et al., 2003). Furthermore, the similarities of human and mouse DISC1 and of human and mouse RASSF7 are 54.76% and 73.08%, respectively. First, we investigated whether there was direct interaction between RASSF7 and RAS and the effect of RASSF7 on the phosphorylation of MEK and ERK. Our co-IP analysis results showed that RASSF7 does indeed interact with RAS (Fig. 6A). We next investigated whether there was an interaction between DISC1 and RASSF7 and whether this interaction is involved in astrogenesis. To address this possibility, we generated recombinant Flag-tagged DISC1, HA-tagged DISC1, Flag-tagged RASSF7 and HA-tagged RASSF7. The results of co-IP showed that DISC1 and RASSF7 directly interact with each other (Fig. 6B,C). This interaction between DISC1 and RASSF7 was further verified by the co-IP of the purified tagged proteins (Fig. S6A). Moreover, colocalization of DISC1 and RASSF7 was observed in cells co-transfected with Flag-tagged DISC1, HA-tagged DISC1, Flag-tagged RASSF7 and HA-tagged RASSF7. The results of co-IP showed that DISC1 and RASSF7 directly interact with each other (Fig. 6B,C). This interaction between DISC1 and RASSF7 was further verified by the co-IP of the purified tagged proteins (Fig. S6A). Moreover, colocalization of DISC1 and RASSF7 was observed in cells co-transfected with Flag-tagged DISC1 and HA-tagged RASSF7 (Fig. S6B), and the binding complex of DISC1-RASSF7 gradually increased as the levels of RASSF7 increased (Fig. S6C). Furthermore, in utero electroporation demonstrated that RASSF7 overexpression enhanced astrocyte differentiation in vivo (Fig. 6D).

How does DISC1 interact with RASSF7? DISC1 contains multiple interaction domains: a globular domain localized in the N-terminus, two leucine zipper domains localized in the middle and C-terminus, and several coiled-coil domains along the full length (Singh et al., 2010). To map the region(s) of DISC1 involved in the association with RASSF7, we generated a series of Flag-tagged fragments of DISC1 and co-expressed them with HA-tagged RASSF7. The results of co-IP showed that DISC1 and RASSF7 directly interact with each other (Fig. 6B,C). This interaction between DISC1 and RASSF7 was further verified by the co-IP of the purified tagged proteins (Fig. S6A). Moreover, colocalization of DISC1 and RASSF7 was observed in cells co-transfected with Flag-tagged DISC1 and HA-tagged RASSF7 (Fig. S6B), and the binding complex of DISC1-RASSF7 gradually increased as the levels of RASSF7 increased (Fig. S6C). Furthermore, in utero electroporation demonstrated that RASSF7 overexpression enhanced astrocyte differentiation in vivo (Fig. 6D).

Fig. 5. DISC1 regulates astrogenesis by modulation of pMEK and pERK levels. (A) Western blot analysis of MEK and ERK. NPCs were infected with DISC1 shRNA2 or shRNA3 lentivirus and cultured in differentiation medium for 3 days. Following serum starvation overnight, cells were treated with LIF (50 ng/µl) for 10 min. The cell lysates were subjected to western blot analysis for phosphorylated and total MEK, and phosphorylated and total ERK. The levels of pMEK and pERK decreased due to DISC1 knockdown. (B) Western blot of NPCs infected with the DISC1 overexpression lentivirus. The levels of pMEK and pERK increased due to DISC1 overexpression in vivo, as demonstrated by GFAP staining. Right: the percentages of GFAP* GFP* cells relative to GFAP* cell numbers (t-test, *P=0.033). Error bars indicate s.e.m. (n=3). Scale bar: 50 μm. See also Fig. S5.

Fig. 4. DISC1 overexpression rescues astrogenesis defects induced by mouse DISC1 depletion. (A,C) Reduced GFAP expression due to mouse DISC1 (mDISC1) knockdown was rescued by human DISC1 (hDISC1) overexpression. Embryonic NPCs were infected with different combinations of lentivirus and cultured for 4 days. (B) Overexpression of hDISC1 leads to more astrocytes in vivo. Left: increased numbers of GFAP-positive cells in P2 cortices of embryos electroporated at E16. Right: the proportion of GFAP*GFP* astrocytes (t-test, **P=0.0094) relative to total GFP* cells. Error bars indicate s.e.m. (n=4). (D) The decrease in astrocyte number caused by mDISC1 knockdown is rescued by a gain of hDISC1 in vivo. Left: confocal images of GFAP immunostaining. Right: the percentage of double-labeled GFAP*GFP* cells relative to GFAP* cells (t-test, *P=0.023). Error bars indicate s.e.m. (n=3). Scale bars: 50 μm. See also Fig. S4.
co-IP results demonstrated that the CT domain of RASSF7 was involved in the association with DISC1 in the complex (Fig. 6F). Furthermore, the co-IP results demonstrated that the CT domain of DISC1 was sufficient to associate with the CT domain of RASSF7 (Fig. 6G). We then constructed DISC1-ΔNT2 or DISC1-ΔMD plasmids and performed rescue experiments to test whether DISC1 mutants could rescue the mDISC1 shRNA effects in vivo. Immunostaining of GFAP showed that the number of astrocytes was increased when DISC1-ΔNT2 and mDISC1 shRNA2 were co-expressed (Fig. S6D). However, the number of GFAP+ cells was not significantly affected when DISC1-ΔMD and mDISC1 shRNA2 were co-expressed (Fig. S6E). These data suggest that the
MD domain, not the NT2 domain, is important for the function of DISC1. Taken together, these results indicate a direct and complex interaction between DISC1 and RASSF7 via multiple domains.

Because RASSF7 belongs to the RAS family, we then investigated whether RAS/MEK/ERK signaling is involved in the process. First, we examined pERK and found that the levels of pERK were increased in the presence of RASSF7 in a dose-dependent manner (Fig. S6F). Moreover, the results showed that the overexpression of RASSF7 or DISC1 alone increased the levels of pMEK and pERK in the presence of LIF; and RASSF7 and DISC1 overexpression resulted in higher levels of pMEK and pERK (Fig. S6G). Interestingly, the overexpression of RASSF7 restored the pMEK and pERK levels caused by the reduction of DISC1 shRNA (Fig. S6H). These data suggest that RASSF7 is required for the DISC1-dependent modulation of MEK/ERK signaling (Fig. 7).

**DISCUSSION**

**DISC1** is one of only a few single genes definitively associated with psychiatric disease, such as schizophrenia, bipolar disorder and unipolar depression (Bradshaw and Porteous, 2012). However, it is unclear how DISC1 dysfunction results in a series of mental disorders. Recently, the important role of DISC1 in the processes of neuronal development has been demonstrated (Mao et al., 2009; Niwa et al., 2010), but its role in astrogenesis is not clear. Furthermore, the etiology of schizophrenia remains to be comprehensively characterized. Thus, we set out to describe the role of DISC1 in astrocyte progenitor differentiation during the late stage of brain development by using in utero electroporation approaches of gene manipulation.

In this study, we found that DISC1 depletion reduces the expression of ACSBG1 and GFAP in vitro and decreases astrocyte number in vivo, whereas DISC1 overexpression substantially enhances the process. Moreover, a missense mutation of DISC1 (DISC1-L100P) constructed according to a previous paper (Clapcote et al., 2007) causes abnormal gliogenesis. In order to test whether the decrease in astrocyte number is the result of changes in proliferation, we performed BrdU-labeling experiments. The results showed that there was no significant difference in BrdU labeling of GFAP+ GFP+ cells by DISC1 knockdown and DISC1 overexpression (Fig. S7A,B). Furthermore, brains electroporated with DISC1 overexpression plasmid at E16 were analyzed at P10 and P16 to examine the longer-term effect of DISC1. The results showed the GFP-labeled cells were slightly increased when DISC1 was overexpressed and mainly migrate to the cortical plate. Compared with the control group, the morphology of GFP-labeled astrocytes in the DISC1 overexpression group was more mature (Fig. S8A,B). In addition, we also checked neural stem cell proliferation and neuron differentiation. E16 embryonic brains electroporated with control and DISC1 knockdown plasmids were analyzed at E18 or P0 (Fig. S8C,D). The results showed that DISC1 knockdown cells mainly located at the VZ/SVZ and a small proportion of cells migrated to the intermediate zone. When brains were electroporated at E16 and analyzed at P0, we found that the number of nestin-positive cells was slightly decreased with DISC1 knockdown or slightly increased with DISC1 overexpression (Fig. S9A,B). These data suggest that DISC1 knockdown may not affect nestin-positive progenitor cell proliferation at late stages of development. We also examined neuron differentiation at a late stage. The results showed that the number of neurons differentiated from isolated P0 NPCs and TUJ1 (TUBB3)+ cells were not significantly affected by DISC1 knockdown or overexpression (Fig. S9C-F).

Gliogenesis occurs during the late stages of embryogenesis after neurogenesis, when NPCs generate more astrocytes and fewer neurons. Specific genes related to astrocyte development are temporally and spatially expressed during late embryonic brain development following the activation of different relevant intrinsic signaling pathways. Among these pathways, the JAK-STAT pathway is important for controlling the onset of astrogenesis (Fan et al., 2005). In addition, the RAS/MEK/ERK signaling pathway has been shown to regulate gliogenesis (Li et al., 2012). Our data indicate that DISC1 regulates astrogenesis by modulating pMEK and pERK levels. Furthermore, consistent with a previous study (Clark et al., 2004), we confirmed that the active form of ERK translocated into the nucleus. Interestingly, DISC1 participates in this pathway by interaction with RASSF7, a member of the RAS-association domain family. A previous study used yeast two-hybrid screening to identify that hRASSF7 interacts with hDISC1. We compared the similarities of protein sequences and found that human DISC1 and mouse DISC1, as well as human and mouse RASSF7, were highly homologous. Based on this information, co-immunostaining was performed and the result showed that mouse DISC1 interacts with RASSF7.

To date, various lines of evidence have revealed multiple signaling pathways that correlate with the intrinsic pathogenesis of schizophrenia (Kyosseva, 2004). Recently, DISC1 was proven to be involved in GSK3β/β-catenin signaling (Mao et al., 2009), AKT/mTOR signaling (Kim et al., 2009) and GABA signaling. Given the variety of proteins that bind DISC1, it evidently plays many roles in the central nervous system via different signaling pathways. Here, we add that DISC1 regulates the RAS/MEK/ERK pathway, which is important for brain astrocyte development and function. The influence of DISC1 on the development of neurons and oligodendrocytes has been investigated (Hattori et al., 2014; Mao et al., 2009). Here, we further investigate the effects of DISC1 on astrocyte development. In summary, our results provide a framework for understanding the effect of DISC1 on astrogenesis, which may contribute to brain development and the etiology of psychiatric disorders.

**MATERIALS AND METHODS**

**Mice**

ICR female mice were used for in utero and postnatal electroporation experiments. For timed breedings, the day after detection of a vaginal plug was considered to be E1. Mice were maintained and bred in the Experiment
Animal Center of Institute of Zoology, Chinese Academy of Sciences. All animal experiments and protocols were approved by the Animal Care and Use Committee of Institute of Zoology, Chinese Academy of Sciences.

**Plasmid constructs**

Lentivirus vectors were used for cloning of plasmid constructs (Duan et al., 2007). See supplementary Materials and Methods for further details.

**Cell culture**

Lentivirus was produced by 293FT and the collected virus used to infect NPCs. NPCs used for immunofluorescence and western blotting were isolated from E15 embryoembryonic brains. See supplementary Materials and Methods for culture conditions.

**Western blotting and co-immunoprecipitation**

Proteins from cortices or cultured primary NPCs were prepared in the RIPA buffer containing 1% protease inhibitor cocktail and 1% phenylmethylsulfonyl fluoride and ultrasonicated on ice. After centrifugation at 12,000 rpm (13,523 g) for 10 min (4°C), protein concentrations were measured using the Pierce BCA Protein Assay Kit (Thermo Scientific). Lysates were size-separated by 12% SDS-PAGE, blotted onto nitrocellulose membrane (Millipore) by the semi-dry electrophoretic transfer according to standard protocols (Bio-Rad) (Lv et al., 2014). For co-IP analysis, fresh samples were immunoprecipitated with anti-Flag or anti-GFP antibodies that had been incubated with Dynabeads Protein A (Life Technology) overnight at 4°C, and then subjected to western blotting according to the manufacturer’s protocols (Life Technology). See supplementary Materials and Methods for details of antibodies used.

**In utero and postnatal electroporation**

Every embryo was electroporated with five 50 ms pulses at 50 V with 950 intervals, using 5 mm paddle electrodes. The embryos were returned to the abdominal cavity 30 min after electroporation (Wang et al., 2014). Neonatal pups were electroporated with four 50 ms pulses at 90 V with a 950 ms interval. See supplementary Materials and Methods for further details.

**Immunohistochemistry and immunocytochemistry**

Brains of mice were fixed in 4% paraformaldehyde (PFA) for 24 h at 4°C, gradually dehydrated in 5%, 10%, 15%, 25%, 30% sucrose for 8 h in sequence, and then embedded in OCT overnight at −20°C. Coronal sections (15 μm) were cut using Leica CM1950 cryostat. For IHC, sections were fixed in 4% PFA for 30 min, washed twice with PBS, blocked with 5% bovine serum albumin in PBS containing 0.1% Triton X-100 for 1 h, and then incubated in primary antibodies overnight at 4°C. The next day, brain slices were washed three times with PBS, and incubated with secondary antibody for 2 h at room temperature. After three washes in PBS, sections were counterstained with DAPI (2 μg/ml). The steps for immunocytochemistry and BrdU labeling were similar to the above protocol (Zhang et al., 2014). See supplementary Materials and Methods for details of antibodies used.

**Statistical analysis**

Statistical analysis was performed using Student’s t-test or one-way ANOVA. All bar graphs are plotted as mean±s.e.m. P-values of <0.05 were considered to be significant (*P<0.05; **P<0.01; ***P<0.001). For details of quantification methods (Barnabé-Heider et al., 2005; Xie et al., 2007), see the supplementary Materials and Methods.

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**Competing interests**

The authors declare no competing or financial interests.

**Author contributions**

S.W., Q.L. and J.J. designed the study, analyzed the date, wrote the paper; S.W. and Q.L. performed all the experiments; H.Q. and T.S. did several of the experiments; H.L. and F.J. helped to conceive the study.

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**Supplementary information**

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