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Research paper

Roles of Reelin/Disabled1 pathway on functional recovery of hemiplegic mice after neural cell transplantation; Reelin promotes migration toward motor cortex and maturation to motoneurons of neural grafts

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ABSTRACT

Reelin is a large glycoprotein which regulates central nervous system (CNS) development. Dysfunctions of Reelin were reported on certain neuropsychiatric diseases. We examined involvement of Reelin pathway in functional recovery of hemiplegic mice after neural transplantation.

Reelin was expressed 1 day after cryogenic injury of right motor cortex. We transplanted neural stem/progenitor cells (NSPCs) from wild-type mice into ipsilateral striatum of hemiplegic mice. The grafts migrated from the striatum and reached the injured cortex 14 days after transplantation. The transplantation significantly improved their motor functions ($P < .05$).

The NSPCs migrating toward the cortex expressed Reelin receptors, Apoer and Vldlr, and phosphorylated Disabled1 (Dab1), a downstream signaling molecule of Reelin. The grafts expressed Ncadherin and active form of Integrin β1, both of which were known to become active with Reelin stimulation. At day 28, the grafts expressed Ctip2, Crim1, Foxp2, and Fezf2, all of which were forebrain motoneuron associated markers, and Nfm and Synapsin1 on the damaged cortex.

We then transplanted NSPCs of *yotari* mice (*yot/yot* genotype) having nonfunctional Dab1 by a mutation of its gene. Majority of the grafts from *yotari* mice (> 80%) did not migrate and thus remained at the striatum. The grafts did not express the forebrain motoneuron associated markers nor the cell adhesion molecules including Ncadherin and active Integrin β1.

Reelin pathway was involved in graft migration by regulating certain adhesion molecules and in their differentiation to functional motoneurons accompanying synapse formation. We suggested involvement of Reelin pathway for neural regeneration and functional recovery of hemiplegic mice in adulthood after neural transplantation.

1. Introduction

CNS developments are governed by several secreted molecules, including Reelin. Much less is elucidated on molecules involved in the functional regeneration of damaged CNS in adulthood.

We transplanted the neural cells of various origins into brain of the hemiplegic mice (Chiba et al., 2003, 2004; Ikeda et al., 2004, 2005; Hazama et al., 2010) where we had induced the hemiplegia by cryogenic injury of right motor cortex in normal mice. We transplanted the cells underneath the injured site, namely center of the dorsal domain of the striatum. The grafted neurons migrated from the striatum into the damaged cortex. They differentiated further accompanying expression of several motoneuron associated markers (Chiba et al., 2003; Ikeda et al., 2005; Suzuki et al., 2017). Moreover, the grafts formed synaptic

Abbreviations: Apoer, apolipoprotein E receptor; bFGF, basic fibroblast growth factor; CMDiI, 3H-Indolium,5-[[[4-(chloromethyl) benzoyl] amino] methyl]-2- [3-(1,3-dihydro-3,3-dimethyl-1-octadecyl-2H-indol-2-ylidene)-1-propenyl]-3,3-dimethyl-1-octadecyl-, chloride; CNS, Central nervous system; Dab1, Disabled1; DAPI, 4,6-diamidino-2-phenylindole; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; Gfap, glia fibrillary acidic protein; MANOVA, multivariate analysis of variance; MEK, mitogen-activated protein kinase; Nfm, neurofilament middle chain; NSPCs, neural stem/progenitor cells; pDab1, phosphorylated Disabled1; Vldlr, very low-density lipoprotein receptor; WT/WT, wild type mice; *yot/yot*, *Yotari* homozygous genotype mice

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vesicles in order to connect neighboring host neurons (Chiba et al., 2004; Ikeda et al., 2004). We previously reported that the grafts migrated in response to stromal cell derived factor (SDF) 1 secreted by glial cells residing around the injured site (Hazama et al., 2010). SDF1 is a chemokine and regulates migration of neurons through its receptor CXCR4 (Arimitsu et al., 2012). SDF1 and CXCR4 interaction activates p38MAPK/c-Jun and induces several cell activities, such as proliferation, structural alterations of the cytoskeleton, and neurite outgrowth.

Reelin is a large glycoprotein and essential for the neural stem cell migration and cortical lamination in the development (D'Arcangelo and Curran, 1998). Its receptors, apolipoprotein E receptor (Apoer) and very low-density lipoprotein receptor (Vldlr), and a cytosolic adaptor protein disabled (Dab) 1 are major components of the Reelin signaling pathway. Phosphorylated Dab1 (pDab1) controlled radial migration and laminar formation of cortical neurons through the phosphorylation of several molecules, such as Akt (Park and Curran, 2008) and Ras associated protein (Rap) 1 (Franco et al., 2011).

In reeler mutant mice, Reelin depletion demonstrated severely disturbed cortical layers in the pathology and ataxic behavior (D'Arcangelo and Curran, 1998). In humans, Reelin gene mutation resulted in autosomal recessive lissencephaly with cerebellar hypoplasia (Hong et al., 2000).

Yotari homozygous mice have a mutated Dab1 gene and show characteristic neurological phenotypes, such as tremors, ataxia, cerebellar hypoplasia, with widespread disruption of cellular layers throughout the brain (Yoneshima et al., 1997), resembling reeler mice. The mutation of Dab1 gene affected major phosphorylation sites, each of which was reported to be required for normal brain development (Howell et al., 2000; Feng and Cooper, 2009; Katyal and Godbout, 2004; Pramatarova et al., 2003). Multipotent neurospheres from the embryonic forebrain of *yotari* mice had tendency to differentiate toward astrocytes rather than neurons (Kwon et al., 2009).

Reelin distribution in the brain was altered remarkably in postnatal period and the expression was observed in GABAergic interneurons localized in the cortex and hippocampus (Alcántara et al., 1998; Pujadas et al., 2010). Reelin pathway was suggested to relate to the modulation of synaptic activity and connectivity in adult mice (Pujadas et al., 2010; Trotter et al., 2013).

Researchers identified disease-associated single-nucleotide polymorphisms of Reelin gene in schizophrenia and autism (Ishii et al., 2016). Reelin expression reduced in postmortem analyses of schizophrenia, bipolar disorder, major depression (Fatemi et al., 2000), and autism (Fatemi et al., 2005). Conditional deletion of Dab1 demonstrated hyperactivity and working memory impairment (Imai et al., 2017). Deficits of Reelin-Dab1 signaling pathway may lead to plaque and tangle accumulation in adult brain, resembling major findings of Alzheimer's disease (Pujadas et al., 2014). These findings indicated multiple roles of Reelin pathway in human neurological and psychiatric disorders.

We here examined roles of Reelin signaling pathway in the neural regeneration and functional recovery of hemiplegic mice after transplantation of neural stem/progenitor cells (NSPCs) derived from wild type mice and *yotari* mice. Our findings support involvement of Reelin signaling pathway to regeneration of motor cortex and subsequent functional recovery in adult hemiplegic mice as well.

2. Materials and methods

2.1. Animals

Female C57 black 6 (C57BL/6) mice (6–8 weeks old, Japan SLC, Shizuoka, Japan) were used as transplant recipients because we wanted to use genetically normal mice.

Yotari strain harbors a *yotari* or *yot* gene mutated at the *Dab1* gene. This mutant arose in the descendants of a male chimeric mouse (chimera of 129 and C57BL/6JJcl) (Yoneshima et al., 1997; Kojima et al., 2000). This strain was provided by Dr. Kazunori Nakajima (Keio University, Tokyo, Japan) through BioResource Center, Riken, Tsukuba, Japan.

All experimental procedures including surgical interventions, pre- and postsurgical animal care, and euthanasia were performed in accordance with the Guide for the Care and Use of Laboratory Animals, 8th Edition (National Research Council) and were approved by the local Animal Care Committee (Animal Care and Use Committee, St. Marianna University School of Medicine).

2.2. Preparation of NSPCs

We collected the neonate from heterozygote intercrosses. The brains of wild type (WT/WT) and *yotari* homozygous (*yot/yot*) mice were dissociated into single cell suspensions. The cells were cultured with epidermal growth factor (EGF, Sigma-Aldrich, catalog # E4127, RRID: SCR_008988) and basic fibroblast growth factor (bFGF, Sigma-Aldrich, catalog # F0291, RRID: SCR_008988) for neurosphere formation (Pacey et al., 2006). The neurospheres were disaggregated into single cell suspensions by TryPLE (Thermo Fisher Scientific, catalog # 12604021, RRID: SCR_008452) and were used for transplantation into hemiplegic mice. Aliquots of the cell suspension were characterized by immunocytochemistry.

2.3. PCR for genotyping of yotari mice

For genotyping of *yot/yot* mice, PCR of specific genomic DNA regions was conducted (Fig. 1*A*). Tail and skin DNA was used as templates (Kojima et al., 2000). The PCR primers used for genotyping were as follows: forward for the fragments: 5′-gcccttcagcatcaccatgct-3′, reverse for the wild mouse fragment, 800 bp: 5′-aaagagatctctcaagtccagg-3′, reverse for the *yotari* mouse fragment, 600 bp: 5′-cagtgagtacatattgtgtgagttcc-3′.

2.4. Experimental brain injury and cell transplantation

Procedures for brain injury and subsequent cell transplantation have been described previously (Chiba et al., 2003, 2004). Briefly, C57BL/6 normal mice were cryoinjured 7 days before transplantation. A burr hole mark was made in the right parietal bone at the location of 0.5 mm anterior and 2.0 mm lateral to the bregma and a metal probe chilled with liquid nitrogen was applied. We transplanted WT/WT NSPCs and *yot/yot* NSPCs into the hemiplegic mice. In order to avoid unnecessary brain injury, we injected through the cryogenic injury site (Chiba et al., 2003, 2004).

A preliminary study disclosed that injection of 5.0×10^5 WT/WT NSPC cells was the most effective for their functional recovery. On the day of cell transplantation (day 0), cell suspension (5.0×10^5 cells in PBS) and vehicle (PBS only) were injected into the ipsilateral periventricular area (Chiba et al., 2003, 2004).

We labeled the NSPCs with 3H-Indolium, 5-[[[4-(chloromethyl) benzoyl] amino] methyl]-2- [3-(1,3-dihydro-3,3-dimethyl-1-octadecyl-2H-indol-2-ylidene)-1-propenyl]-3,3-dimethyl-1-octadecyl-, chloride (CellTracker CMDiI, Molecular Probes, catalog # C-7000, RRID: SCR_013318) (Matsubayashi et al., 2008; Zhao et al., 2012) before transplantation to trace the cells. There were no obvious differences of cell labeling efficiency and cell viability between WT/WT derived NSPCs and *yot/yot* derived NSPCs.

2.5. Motor function analyses

Recovery of motor function was evaluated by beam walking and rotarod tests as previously described (Chiba et al., 2003, 2004). Briefly, mice were trained before and after brain injury to walk along a narrow wooden beam 10 mm wide and 120 mm in length, at the height of 300 mm. The number of foot faults for contralateral (left) hindlimb was

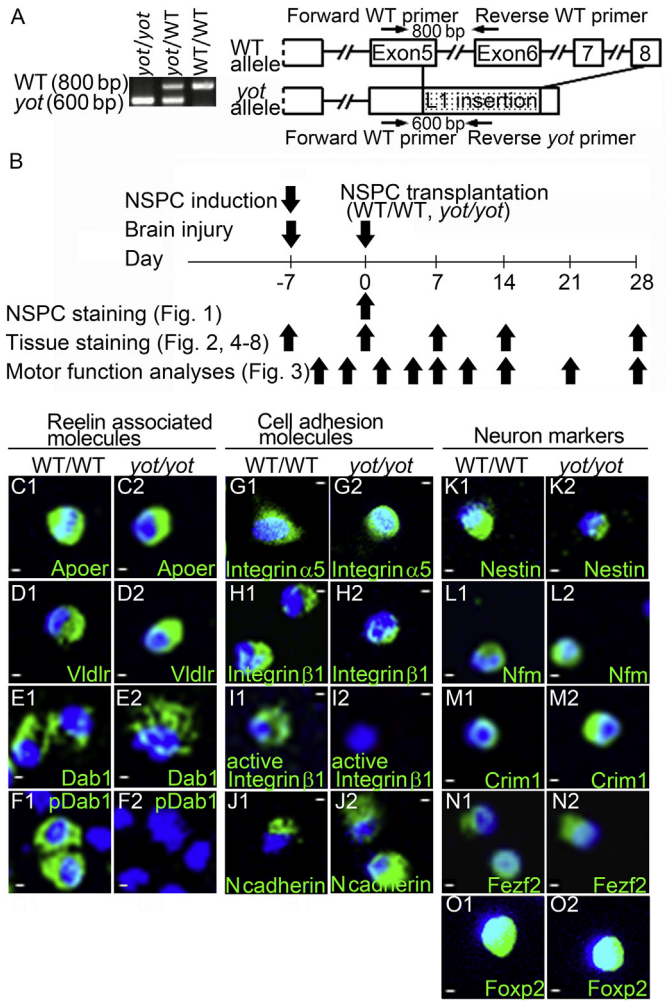

Fig. 1. *A,* Schematic representation of the Dab1 gene structure in WT/WT and *yot/yot* mice. Dab1 gene was partially replaced by the long interspersed nuclear element (L1) fragment in *yot/yot* mice. The amplicon obtained from *yot* allele (forward WT primer to reverse *yot* primer) is 600 bp long and that of WT allele (forward WT primer to reverse WT primer) is 800 bp long. *B,* Experimental schedule of NSPC induction from WT/WT and *yot/yot* mice and subsequent transplantation.

We obtained WT/WT and *yot/yot* neurospheres from neonatal brains at day −7 by culturing with epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). After harvest, the neurospheres were disaggregated into single cell suspensions at day 0 as NSPCs. The single cell suspensions were labeled with CMDil before transplantation to trace the cells.

C–O, Aliquots of the single cell suspensions used for transplantation were stained with the following antibodies, *C,* Apoer, *D,* Vldlr, *E,* Dab1, and *F,* pDab1, cell adhesion molecules, namely *G,* Integrin α5, *H,* Integrin β1, *I,* active Integrin β1, and *J,* Ncadherin, and neuron markers, namely *K,* Nestin, *L,* Nfm, *M,* Crim1, *N,* Fezf2, and *O,* Foxp2. Scale bar indicates 5 μm for *C* through *O*.

WT/WT NSPCs expressed pDab1 and active Integrin β1 and *yot/yot* NSPCs did not express these proteins.

recorded over 50 steps.

Rotarod test was used to assess refined motor function and coordination as previously reported (Chiba et al., 2003, 2004). The mice were placed on the rolling rod at 30 rpm for the maximum of 300 s after basic training of the mice before cryogenic injury. The system recorded the total running time on the rod, the time of fall, and other experimental setup parameters.

2.6. Immunofluorescence staining

Immunofluorescence staining was conducted as reported previously (Chiba et al., 2003, 2004).

Cells and brain sections were stained with antibodies to various mouse molecules as follows: mouse anti-Reelin (1:500, Millipore, catalog # MAB5364, RRID: AB_2179313), rabbit anti-Apoer (1:100, Novus, catalog # NB100–2216, RRID: AB_10002890), goat anti-Vldlr (1:20, R and D Systems, catalog # AF2258, RRID: AB_2288612), rabbit anti-Dab1 (1:100, Abcam, catalog # ab111684, RRID: AB_10865153), rabbit anti-pDab1 (phosphorylated at Y220, 1:100, Santa Cruz Biotechnology, catalog # sc-133,292, RRID: AB_2089596), rabbit anti-Integrin α5 (1:1000, Millipore, catalog # AB1921, RRID: AB_2128176), goat anti-Integrin β1 (1:25, R and D Systems, catalog # AF2405, RRID: AB_416591), rat anti-active Integrin β1 (1:50, BD Biosciences, catalog # 550531, RRID: AB_393729), rabbit anti-Ncadherin (1:100, Abcam, catalog # ab18203, RRID: AB_444317), mouse anti-Nestin (1:100, Millipore, catalog # MAB5326, RRID:AB_2251134), rabbit anti-neurofilament middle chain (Nfm, 1:200, Millipore, catalog # AB1987, RRID: AB_91201), rabbit anti-Crim1 (1:100, Boster, CA, USA, catalog # A06053), rabbit anti-Fezf2 (1:100, Abcam, catalog # ab69436, RRID: AB_10672324), rabbit anti-Foxp2 (1:400, Abcam, catalog # ab16046, RRID: AB_2107107), rabbit anti-glia fibrillary acidic protein (Gfap, 1:4000, Dako, catalog # Z0334, RRID: AB_10013382), rat anti-Ctip2 (1:50, Abcam, catalog # ab18465, RRID: AB_2064130), and rabbit anti-Synapsin1 (1:500, Millipore, catalog # AB1543P, RRID: AB_90757), with appropriate secondary antibodies (Alexa Fluor 488, Invitrogen, RRID: SCR_008452).

For nucleus staining, we used 4′, 6-diamidino-2-phenylindole (DAPI, 0.4 μg/ml in PBS, Sigma, catalog # D9564, RRID: SCR_008988).

For a control staining, we stained the adjacent tissue samples with the same procedure without addition of primary antibodies.

2.7. Statistical analysis

A multivariate analysis of variance (MANOVA) was performed to determine differences among the 4 groups (injured mice with WT/WT NSPC transplantation, injured mice with *yot/yot* NSPC transplantation, injured mice with PBS injection, and mice without brain injury) on beam walking test and rotarod test. Mann-Whitney *U* test was conducted for comparison between groups in immunocyte- and immunohistochemistry. We found accumulation of the grafted NSPCs mainly around the injured cortexes, and took photos of the injured cortex having cell accumulation. We first identified the slices of 30 μm thick from nearly 0.5 mm anterior to the bregma which represented center of the injury site. We then selected consecutive 3 slices of each brain and the numbers of CMDil labeled cells were counted. We found that 70 ± 3.0% of CMDil positive cells migrated across the lesion boundary in the cortexes of WT/WT NSPC transplanted mice, and the remaining CMDil positive cells kept around the grafted site.

All data of the current study were expressed as mean ± standard error of the mean (SEM, $n \geq 6$). Statistical analyses were performed using JMP 13.0.0 software (SAS Institute Japan, Tokyo, Japan). *P* value < .05 was considered significant.

3. Results

We have reported recovery of motor functions of hemiplegic mice after transplantation of NSPCs derived from mouse ES cells (Chiba et al., 2003, 2004; Ikeda et al., 2004), cynomolgus monkey ES cells (Ikeda et al., 2005; Hazama et al., 2010), and human iPS cells (Arimitsu et al., 2012; Kono et al., 2013; Suzuki et al., 2017).

We here examined the downstream signaling molecules of Reelin in order to elucidate the molecular mechanisms governing the histological regeneration and functional recovery of the hemiplegic mice after transplantation of NSPCs.

Table 1
Percentages of the cells expressing Reelin associated molecules *in vitro* in WT/WT NSPCs and in *yot/yot* NSPCs at the time of transplantation.

Origin of NSPCs		
Molecules	WT/WT	*yot/yot*
Apoer	95.2 ± 4.7%	93.1 ± 3.7%
Vldlr	70.0 ± 13%	64.6 ± 14%
Dab1	96.1 ± 2.2%	91.0 ± 5.5%
pDab1	22.2 ± 2.5%	0.0 ± 0.0%*
Integrin α5	68.2 ± 3.6%	48.2 ± 12%*
Integrin β1	59.6 ± 8.5%	50.4 ± 11%
active Integrin β1	8.5 ± 6.9%	0.0 ± 0.0%*
Ncadherin	79.5 ± 8.0%	54.0 ± 7.9%*
Crim1	48.7 ± 10%	40.4 ± 8.9%
Fezf2	45.4 ± 8.9%	33.8 ± 12%
Foxp2	53.7 ± 15%	37.3 ± 11%
Nestin	87.8 ± 8.1%	80.9 ± 6.1%
Nfm	55.7 ± 12%	47.8 ± 11%

* Significant differences were shown between WT/WT and yot/yot NSPCs ($n \geq 5$) in the percentages of the indicated antigen positive cells to nucleated cells using Mann-Whitney *U* test ($P < .05$).

3.1. Induction of the NSPCs for transplantation

We conducted preliminary experiments where several cell types and cell culture conditions were tested to induce neurons, which subsequently brought about functional recovery of the hemiplegic mice after transplantation (Chiba et al., 2003, 2004; Kono et al., 2013; Suzuki et al., 2017). We found that the cells derived from neurospheres (Pacey et al., 2006) were most suitable for the current transplantation experiments.

Neurospheres were induced from the neonatal brain of WT/WT mice and *yot/yot* mice at day −7 (Fig. 1*B*). At day 0, the neurospheres were disaggregated and the resulting single cells as NSPCs were transplanted to the brain of hemiplegic mice.

Aliquots of the WT/WT NSPCs and *yot/yot* NSPCs were characterized by immunocytochemistry (Fig. 1*C–P*, Table 1). Percentage of cells expressing pDab1 protein was 22.2 ± 2.5% of the whole WT/WT NSPCs. *yot/yot* NSPCs did not express pDab1 at all (Fig. 1*G*). Percentage of NSPCs expressing active Integrin β1 protein of the whole WT/WT NSPCs was 8.5 ± 6.9%. *yot/yot* NSPCs did not express active Integrin β1 (Fig. 1*J*).

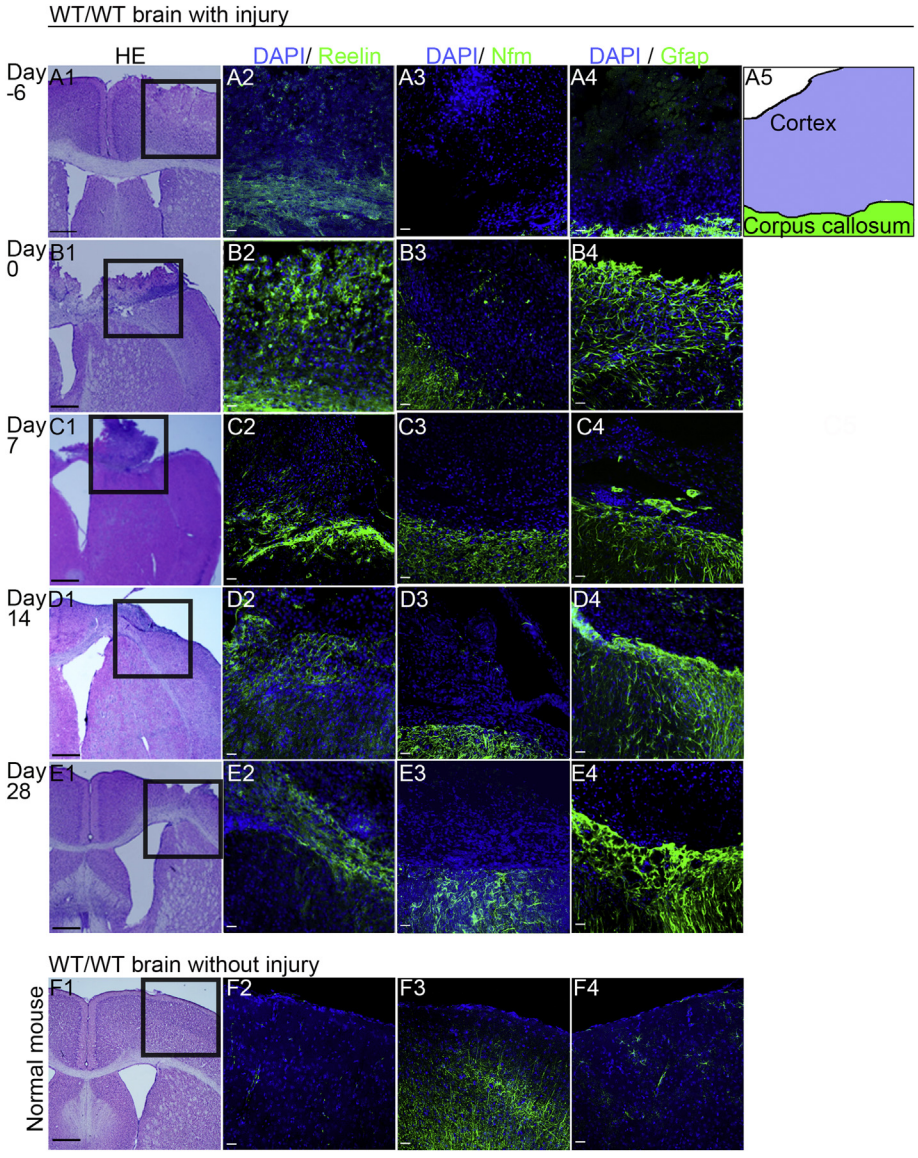

Fig. 2. Reelin expression in the damaged motor cortex of hemiplegic WT/WT mice without neural transplantation.
A, 1 day after brain injury (day −6), *B,* on the day of cell transplantation (day 0), *C,* 7 days after transplantation (day 7), *D,* 14 days after transplantation (day 14), *E,* 28 days after transplantation (day 28). *F,* Reelin expressions in the motor cortex of WT/WT mice without brain injury.
Panels of the first vertical column showed representative HE staining of the injured brain.
The second column showed anti-Reelin staining (green) counterstained with DAPI (blue).
The third column showed anti-Nfm staining (green) counterstained with DAPI (blue). The fourth column showed anti-Gfap staining (green) counterstained with DAPI (blue).
Panel *A5* was a schematic representation of panel *A4*. Panels to the right showing immunohistochemical staining exhibited magnified views of consecutive tissue slice of the corresponding HE staining panel inset to the left.
A, Reelin and Gfap were weakly expressed and Nfm was not expressed on the damaged motor cortex at day −6.
B, C, Reelin and Gfap expressing cells were observed in moderate amounts at day 0 and the numbers of them increased at day 7.
D, Their Reelin expression decreased at day 14.
E, Reelin expression on damaged motor cortex subsided whereas Gfap positive cells sustained moderate abundance at day 28.
F, Motor cortex without injury exhibited markedly lower Reelin expressions.
Scale bar in *A1-F1* indicates 500 μm for panels of the first column; scale bar in *A2-F4* indicates 20 μm for the second - fourth columns. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

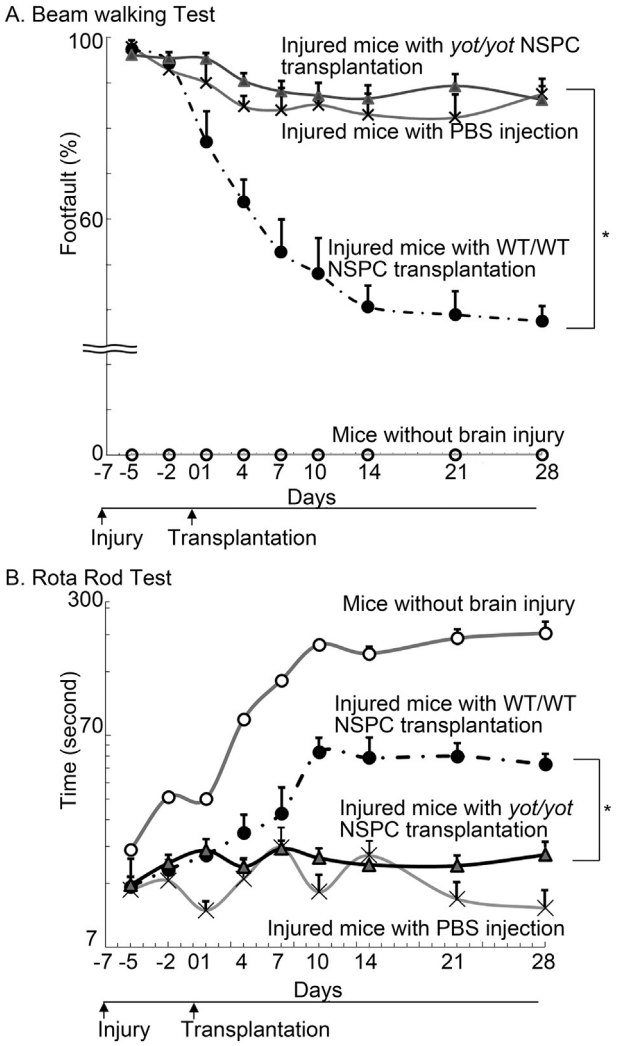

Fig. 3. Analyses of motor functions of hemiplegic mice after the transplantation.

A, beam walking test and *B,* rotarod test conducted from day 1 to day 28 to evaluate motor functions of hemiplegic mice with WT/WT mouse derived grafts (n = 10), those with *yot/yot* mouse derived grafts (n = 18), vehicle (PBS only; n = 16) and control normal mice (n = 6).

Motor functions of mice with WT/WT derived grafts improved significantly compared with those of mice with *yot/yot* derived grafts in both tests. Asterisks indicated that the differences were statistically significant ($P < .05$).

3.2. Expression of Reelin in the damaged motor cortex of hemiplegic mice without NSPC transplantation

We examined whether endogenous Reelin was expressed in the damaged motor cortex (Fig. 2).

At day −6 (1 day after the cryoinjury), Reelin and Gfap were weakly expressed and Nfm was not significantly expressed in the damaged motor cortex (Fig. 2*A2-4*). Reelin and Gfap expressing cells were observed in moderate amounts at day 0 and such the cells increased at day 7 (Fig. 2*B, C*). Expression of Reelin in the damaged motor cortex elicited gradual reduction at day 14 (Fig. 2*D2*). At day 28, the expression of Reelin decreased to some extent whereas Gfap positive cells sustained moderate abundance (Fig. 2*E2,4*). It is possible that Gfap expressing cells were somehow associated with Reelin producing cells in the damaged cortex. We found that βIIITubulin positive neurons produced Reelin on the damaged cortex (data not shown). Motor cortex without injury scarcely had Reelin and Gfap expressions (Fig. 2*F*).

3.3. Improved motor functions of the hemiplegic mice with transplantation of WT/WT NSPCs

We performed beam walking test (Fig. 3*A*) and rotarod test (Fig. 3*B*) from day −5 to day 28 to evaluate motor functions of hemiplegic mice with and without NSPC transplantation. The motor functions of the hemiplegic mice having WT/WT NSPC transplantation improved significantly compared with those of *yot/yot* NSPC transplanted mice. The motor functions of *yot/yot* derived NSPC grafted mice improved marginally when compared with those of PBS injected mice.

The mortality rates were 4.0% and 1.0% in all injured mice within the day before the transplantation and after the transplantation, respectively. The mortality rates and weight changes did not differ significantly among the three mouse groups (WT/WT NSPC transplanted, *yot/yot* NSPC transplanted, and PBS injected mice).

3.4. Directed migration of the transplanted NSPCs toward the injured motor cortex

We conducted histological analyses of injured brains after transplantation of WT/WT and *yot/yot* NSPCs (Fig. 4). The cell aggregates of WT/WT NSPCs detected by hematoxylin-eosin (HE) staining localized at the striatum injection site at day 4 (Fig. 4*A2*). The transplanted WT/WT NSPCs migrated upwards from the transplantation site, approaching to the corpus callosum at day 7, then crossing it at day 14 (Fig. 4*B,C*). At day 28, WT/WT NSPCs were distributed widely around the damaged motor cortex (Fig. 4*D,E*). We used CMDiI which labels NSPCs a fluorescent red, to determine cell localization.

The percentages of CMDil positive WT/WT cells in the whole nucleated cells (DAPI positive cells) in the damaged cortex increased substantially throughout the observation period (Fig. 4*E1*). The percentages of WT/WT cells in the whole nucleated cells in the injected striatum decreased substantially throughout the observation period (Fig. 4*E2*). The percentages of CMDil positive *yot/yot* cells of the whole nucleated cells in the damaged cortex remained low throughout the observation period (Fig. 4*E1*). The percentages of CMDil positive *yot/yot* cells of the whole nucleated cells in the injected striatum remained relatively high throughout the observation period (*E2*). These results suggested that *yot/yot* NSPCs exhibited poor migration capability. We did not find the grafted neurons in the injured cortex at day 7 in both mice with WT/WT NSPC transplantation and those with *yot/yot* NSPC transplantation.

3.5. Expressions of Reelin downstream signaling molecules after NSPC transplantation

We examined the expressions of Reelin downstream signaling molecules in the cortex grafted with WT/WT NSPCs, in comparison with those in the cortex grafted with *yot/yot* NSPCs (Fig. 5).

At the injection site of striatum, grafted cells derived from WT/WT and *yot/yot* NSPCs expressing Apoer (Fig. 5*A*6), Vldlr (Fig. 5*B*6), and Dab1 (Fig. 5*C*6) existed at day 7 and decreased at day 14 and at day 28.

In the striatum, grafted cells derived from WT/WT NSPCs expressing pDab1 existed at day 7, those having accelerated expression were found at day 14, and mostly disappeared at day 28 (Fig. 5*D*6). However, in the striatum, grafted cells derived from *yot/yot* NSPCs expressing pDab1 were scarcely present throughout the observation period.

In the cortex, 68.3 ± 6.2% of grafted WT/WT NSPCs expressed pDab1 at day 14 (Fig. 5*D*5), suggesting that Reelin/Dab1 pathway played a role in the cell migration and differentiation. Grafted cells derived from *yot/yot* NSPCs expressing these molecules were rarely observed throughout the observation period in the cortex (Fig. 5*A-D6*).

3.6. Expressions of cell adhesion molecules on the grafted cells

Expression and functional activation of several cell adhesion

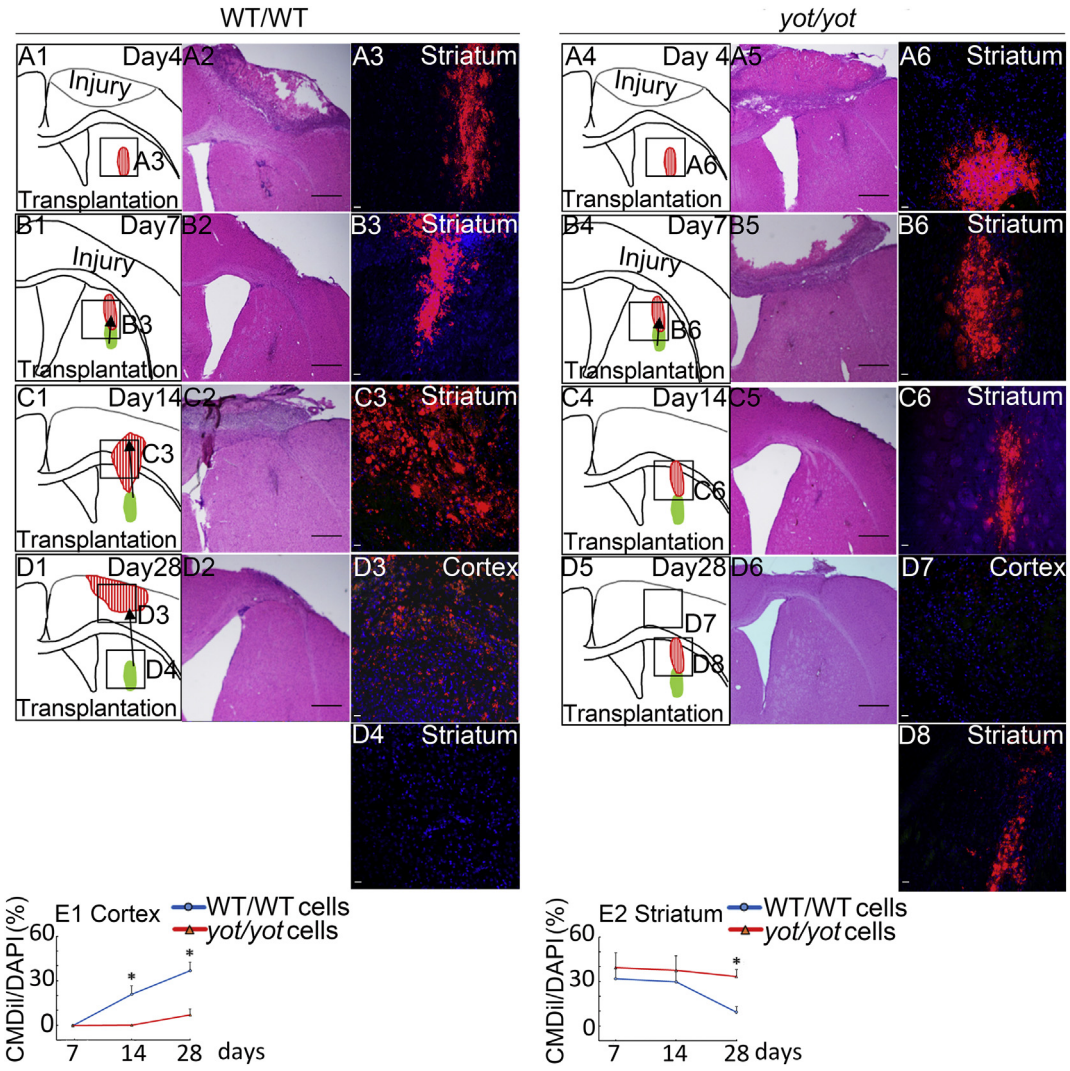

Fig. 4. Distribution of the grafted NSPCs by using HE staining and a fluorescent cell tracker dye on the injured brains.

A, 4 days after transplantation (day 4), *B,* 7 days after transplantation (day 7), *C,* 14 days after transplantation (day 14), and *D,* 28 days after transplantation (day 28). The first and fourth columns showed schematic illustrations of HE staining of mouse brains grafted with WT/WT derived NSPCs and those with *yot/yot* derived NSPCs, respectively. The injection site was shown by green oval area and the grafted cell aggregate was indicated by an area of the red thin stripe pattern. Arrows indicated migration of the grafted cells.

The second and fifth columns showed HE staining of the brains grafted with WT/WT derived NSPCs and those with *yot/yot* derived NSPCs, respectively.

The third and sixth columns showed fluorescence analyses of NSPCs labeled with a cell tracker on brains grafted with WT/WT derived NSPCs and those with *yot/yot* derived NSPCs, respectively.

A, The cell aggregates of WT/WT derived graft localized at the striatum injection site at day 4.

B, C, The cells of WT/WT derived graft moved from the striatum to upward, approaching to the corpus callosum at day 7, then crossing it at day 14.

D, At day 28, the cells of WT/WT derived graft distributed widely over the damaged motor cortex. The cells of *yot/yot* derived graft showed marginal migration and a vast majority of them remained at the injected striatum.

Panels *E1* and *E2* showed percentages (Mean + SEM of 6 mice) of the cells labeled with CMDil to the total nucleated cells (DAPI positive) in the injured cortex and injected striatum, respectively.

Scale bar in panel *A2-D2, A5-C5, D6* indicates 500 μm for panels of the second and fifth columns; scale bar in *A3-D4, A6-D8* indicates 20 μm for the third and sixth columns.

Asterisks indicated that the percentages were significantly higher in the relevant mouse group than the other mouse group ($P < .05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

molecules, such as Integrin α5β1, and Ncadherin, are known to be controlled by an intracellular signaling pathway downstream of Reelin (Franco et al., 2011). The cell adhesion molecules may contribute to migration of the grafted cells, leading to recovery of motor function in the hemiplegic mice. We thus examined expressions of the cell adhesion molecules of the grafts in the damaged cortex of hemiplegic mice with WT/WT derived NSPC transplantation and those with *yot/yot* derived NSPC transplantation (Fig. 6).

At the injection site of striatum, grafted cells derived from WT/WT and *yot/yot* NSPCs expressing Integrin α5 (Fig. 6*A*11) and β1 (Fig. 6*B*11) existed at day 7 and decreased at day 14 and at day 28.

In the striatum, grafted cells derived from WT/WT NSPCs expressing active Integrin β1 and Ncadherin existed at day 7 and decreased at day 14, and mostly disappeared at day 28 (Fig. 6*C,D*11). In the striatum, grafted cells derived from *yot/yot* NSPCs expressing active Integrin β1 and Ncadherin were present in lower percentages throughout the observation period (Fig. 6*C,D*11).

In the cortex, we observed WT/WT NSPC derived grafted cells

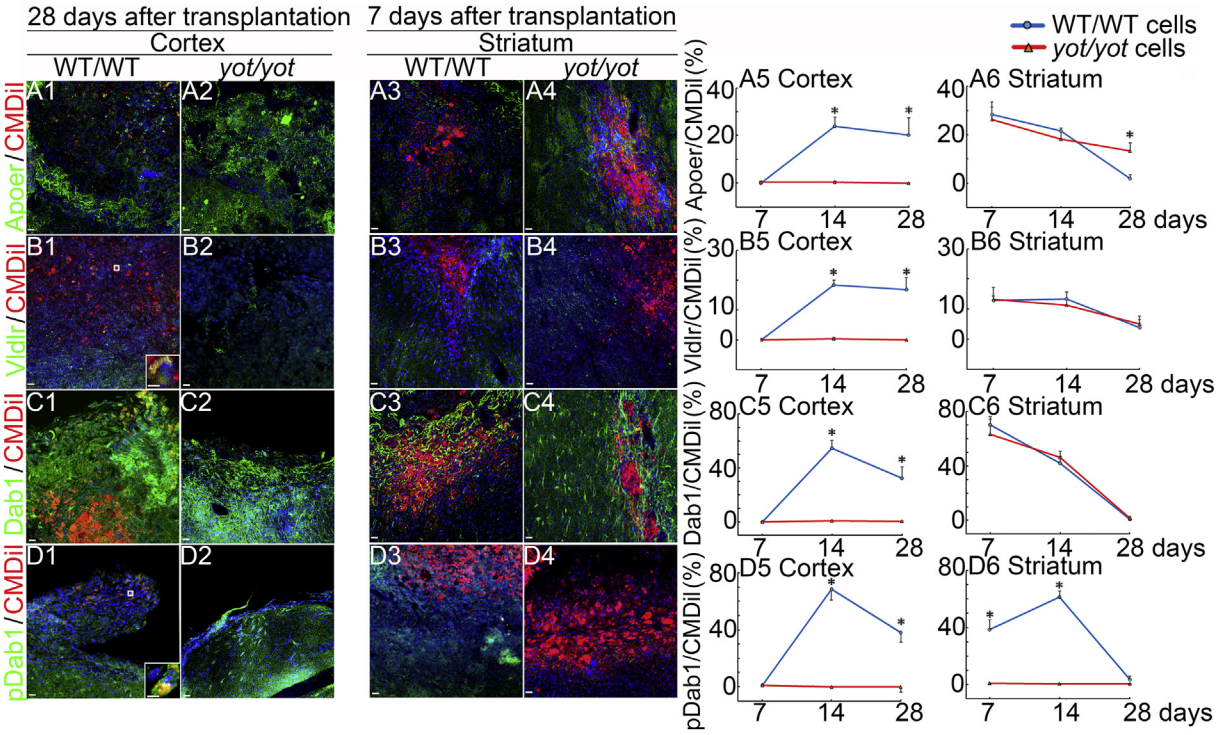

Fig. 5. Expressions of Reelin downstream signaling molecules in the damaged motor cortex and the injected striatum after transplantation.
A, Anti-Apoer (green), CMDil (red), DAPI (blue).
B, Anti-Vldlr (green), CMDil (red), DAPI (blue). The small right lower panel of *B1* was the magnified view of *B1* inset.
C, Anti-Dab1 (green), CMDil (red), DAPI (blue).
D, Anti-pDab1 (green), CMDil (red), DAPI (blue). The small right lower panel of *D1* was the magnified view of *D1* inset.
Panels *A5-D5* and *A6-D6* showed percentages of cells expressing the markers to CMDil labeled cells in the injured cortex and the injected striatum, respectively.
WT/WT NSPCs expressing pDab1 increased until day 14 and mostly disappeared at day 28.
Scale bar in the panel indicates 20 μm except insets of panels *B1* and *D1* (5 μm).
Asterisks indicated that the percentages were significantly higher in the relevant mouse group than the other mouse group ($P < .05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

expressing Integrin α5 (Fig. 6*A*10), β1 (Fig. 6*B*10), active Integrin β1 (Fig. 6*C*10), and Ncadherin (Fig. 6*D*10) at day 28.

3.7. Expression of cortical motoneuron associated proteins on the grafted neurons

We examined expression patterns of molecules associated with cortical motoneurons, namely Ctip2, Crim1, Foxp2, and Fezf2, to estimate the maturity of the grafted NSPCs (Fig. 7).

The motoneuron associated marker expressing grafted cells increased in the hemiplegic mouse cortex with transplantation of WT/WT derived NSPCs. Crim1 expressing cells increased from day 7 to day 14 in the injured cortex (Fig. 7*B*). Ctip2 expressing cells and Fezf2 expressing cells increased substantially from day 7 to day 28 in the injured cortex (Fig. 7*A,D*). Such motoneuron associated marker expressing cells were scarcely detected in the hemiplegic mouse cortex grafted with *yot/yot* derived NSPCs.

3.8. Expression of synapse associated proteins on the grafted neurons

We then evaluated the expressions of Nfm and Synapsin1 in the damaged motor cortex following NSPC transplantation to observe the maturation of NSPCs (Fig. 8).

In the damaged cortex, grafted WT/WT NSPCs expressed Nfm and Synapsin1 and grafted *yot/yot* NSPCs did not expressed these proteins at day 28. In the injected striatum, grafted *yot/yot* NSPCs marginally expressed Nfm and did not express Synapsin1 at day 28.

4. Discussion

In the embryonic development, Reelin is secreted by Cajal-Retzius cells in the marginal zone of the hippocampus and cortex (D'Arcangelo and Curran, 1998). Neural stem cells from the subventricular zone of the cerebrum migrate toward the marginal zone in response to Reelin signaling. Reelin exerts its activities through binding to its receptors, Apoer and Vldlr. Dab1, a cytosolic adaptor protein, binds to the receptors and is phosphorylated by Src family kinases or Fyn, as the result of Reelin stimulation (Arnaud et al., 2003; Bock and Herz, 2003). Phosphorylated Dab1 then recruits Crk and CrkL, which are coupled with C3G, a Rap1 guanidine nucleotide exchange factor (Ballif et al., 2004). The pathway activates Rap1, a small G-protein, which is suggested to promote neuronal locomotion and translocation through the expressions of Ncadherin (Franco et al., 2011; Jossin and Cooper, 2011) and Integrin α5β1 (Sekine et al., 2012).

Reelin and its downstream signaling molecules play an important role in brain maintenance in the adults as well. Reelin promotes neuron dendrite outgrowth through the receptors (Apoer and Vldlr), Dab1, and PI3K/Akt pathway (Pujadas et al., 2010; Trotter et al., 2013). Reelin modulates neuron synaptic function through the receptors (Apoer and Vldlr), Dab1, and mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway (Brai et al., 2015; Trotter et al., 2013). Reelin signaling pathway is essential for neural stem cell migration through the receptors (Apoer and Vldlr), Dab1, C3G, Rap1, and the downstream cell adhesion molecules, Ncadherin and Integrin α5β1 (Franco et al., 2011).

Reeler mutant mice showed that Reelin depletion severely disturbed

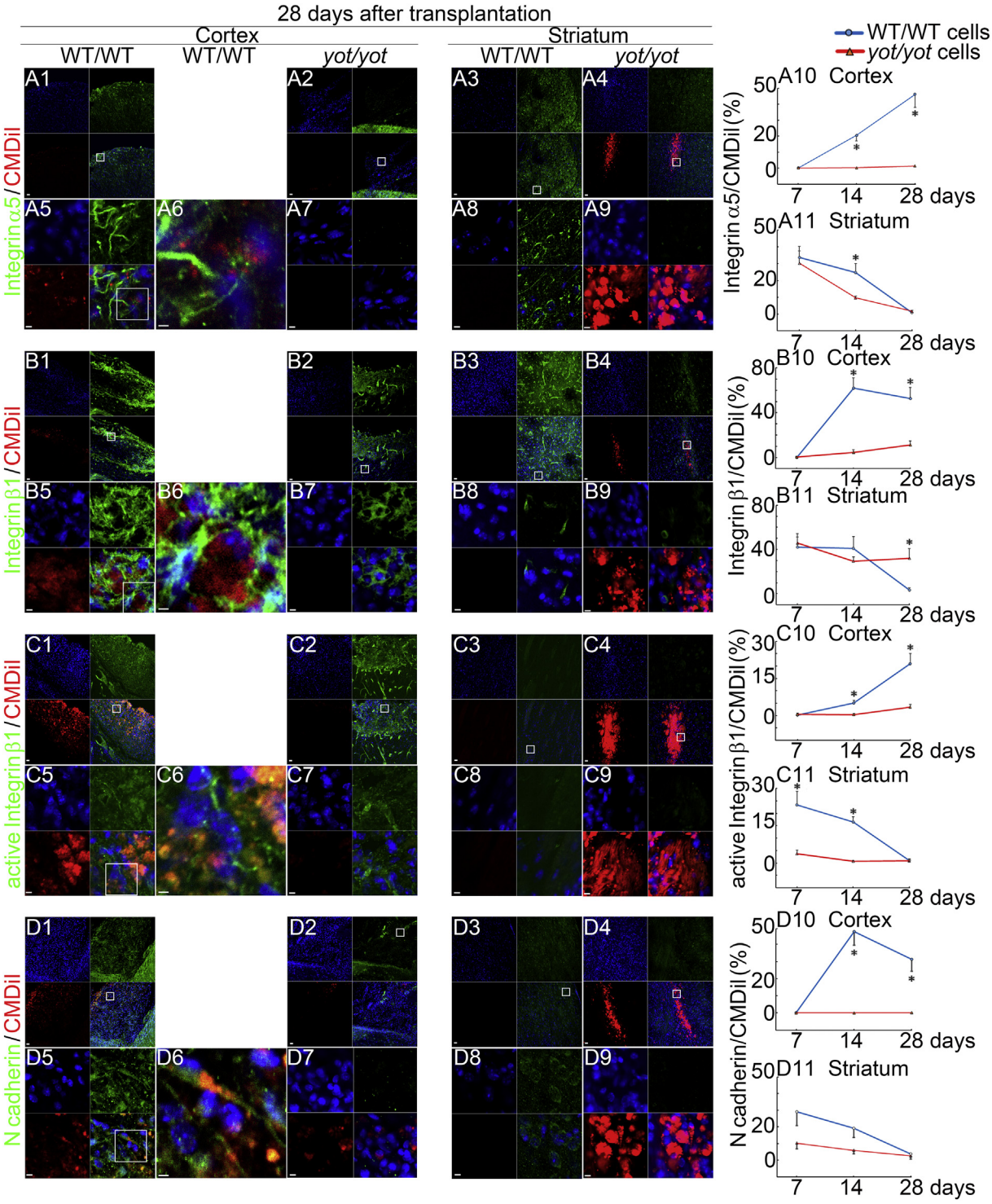

Fig. 6. Expressions of cell adhesion molecules on the grafted cells in the damaged motor cortex and the injected striatum at day 28.
A, Anti-Integrin α5 (green), CMDil (red), DAPI (blue).
B, Anti-Integrin β1 (green), CMDil (red), DAPI (blue).
C, Anti-active Integrin β1 (green), CMDil (red), DAPI (blue).
D, Anti-Ncadherin (green), CMDil (red), DAPI (blue).
Panels *A5-D5*, *A6-D6*, *A7-D7*, *A8-D8*, and *A9-D9* were magnified views of *A1-D1*, *A5-D5*, *A2-D2*, *A3-D3*, and *A4-D4* insets, respectively.
Panels *A10-D10* and *A11-D11* showed percentages of cells expressing the markers to CMDil labeled cells in the injured cortex and the injected striatum, respectively.
WT/WT NSPC derived grafted cells expressing integrin α5, β1, active Integrin β1, and Ncadherin existed at day 28 in the injured cortex.
Scale bar in panel *A1–4, B1–4*, *C1–4*, and *D1–4* indicates 20 μm. Scale bar in panel *A5, A7–9*, *B5*, *B7–9*, *C5*, *C7–9*, *D5*, and *D7–9* indicates 5 μm. Scale bar in panel *A6, B6*, *C6*, and *D6* indicates 2 μm.
Asterisks in the graphs indicated that the percentages were significantly higher in the relevant mouse group than the other mouse group ($P < .05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

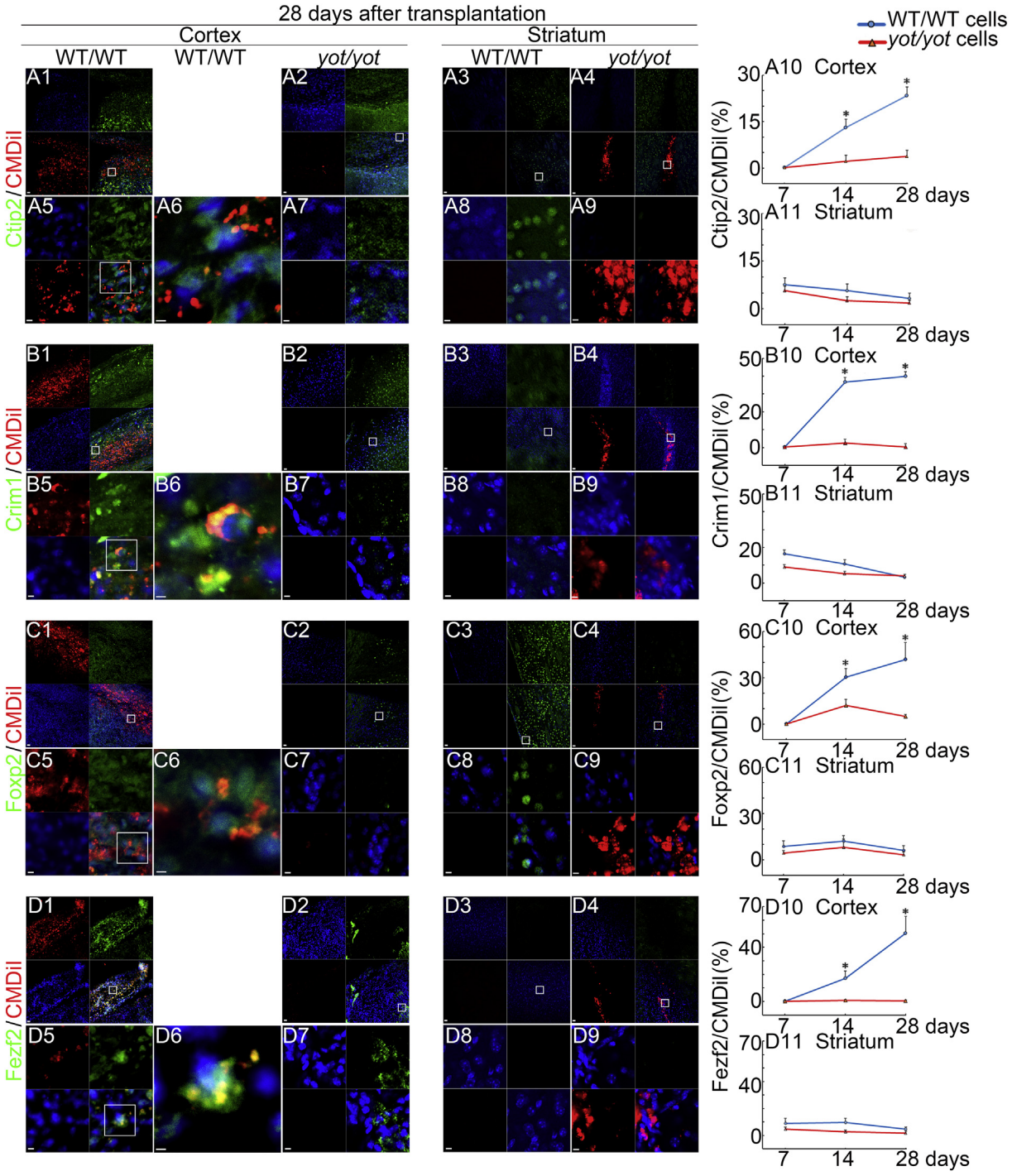

Fig. 7. Expressions of motoneuron associated markers on the grafted cells in the damaged motor cortex and the injected striatum at day 28.
A, Anti-Ctip2 (green), CMDil (red), DAPI (blue).
B, Anti-Crim1 (green), CMDil (red), DAPI (blue).
C, Anti-Foxp2 (green), CMDil (red), DAPI (blue).
D, Anti-Fezf2 (green), CMDil (red), DAPI (blue).
Panels *A5-D5*, *A6-D6*, *A7-D7*, *A8-D8*, and *A9-D9* were magnified views of *A1-D1*, *A5-D5*, *A2-D2*, *A3-D3*, and *A4-D4* insets, respectively.
Panels *A10-D10* and *A11-D11* showed percentages of cells expressing the markers to CMDil labeled cells in the injured cortex and injected striatum, respectively.
The motoneuron associated marker expressing grafted cells increased in the hemiplegic mouse brains with WT/WT derived NSPCs. Such cells were scarcely detected in the hemiplegic mouse brains grafted with *yot/yot* derived NSPCs.
Scale bar in panels *A1–4*, *B1–4*, *C1–4*, and *D1–4* indicates 20 μm. Scale bar in panels *A5, A7–9*, *B5*, *B7–9*, *C5*, *C7–9*, *D5*, and *D7–9* indicates 5 μm. Scale bar in panels *A6, B6*, *C6*, and *D6* indicates 2 μm.
Asterisks in the panels indicated that the percentages were significantly higher in the relevant mouse group than the other mouse group ($P < .05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

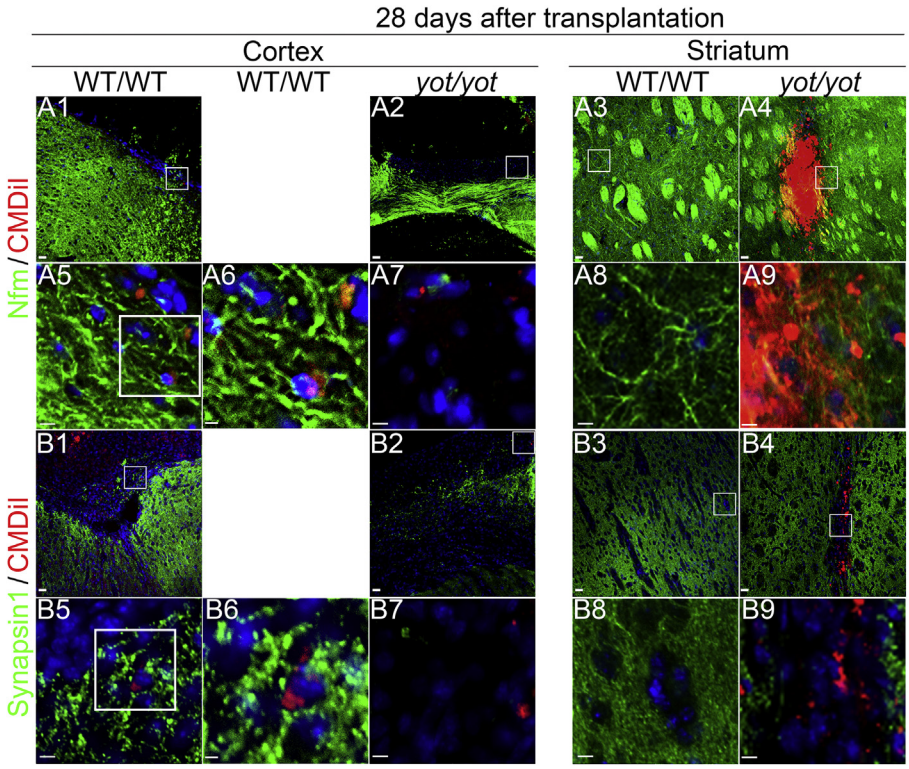

Fig. 8. Expressions of neuron and synapse associated markers on the grafted cells in the damaged cortex and the injected striatum at day 28.
A, Anti-Nfm (green), CMDil (red), DAPI (blue).
B, Anti-Synapsin1 (green), CMDil (red), DAPI (blue).
Panels *A5-B5*, *A6-B6*, *A7-B7*, *A8-B8*, and *A9-B9* were magnified views of *A1-B1*, *A5-B5*, *A2-B2*, *A3-B3*, and *A4-B4* insets, respectively.
In the damaged cortex, grafted WT/WT NSPCs expressed Nfm and Synapsin1 and grafted *yot/yot* NSPCs did not express these proteins at day 28.
Scale bar in panels *A1–4* and *B1–4* indicates 20 μm. Scale bar in panels *A5, A7–9*, *B5*, and *B7–9* indicates 5 μm. Scale bar in panels *A6* and *B6* indicates 2 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cortical layer organization in the brain pathology (D'Arcangelo and Curran, 1998). 2 weeks after the birth, the homozygous reeler mice demonstrated ataxia, imbalance, and reeling gaits (D'Arcangelo and Curran, 1998). *Yotari* mice, which carried the Dab1 gene mutations, showed widespread disruption of cellular layers throughout the brain and did not survive to adulthood (Yoneshima et al., 1997).

We found that the transplanted WT/WT NSPCs along with their maturation, expressed pDab1, Ncadherin, Integrin α5β1, and several motoneuron associated markers. The grafted *yot/yot* NSPCs did not express these molecules or their expression were severely reduced in hemiplegic mouse brains even one month after transplantation. The reduction (in percentage) of the respective Apoer, Vldlr and Dab1 expressing grafts to whole transplanted cells (labeled with the red colour) from day 7 to day 28 was evident in the striatum of *yot/yot* derived NSPC grafted mice.

We found that the injured cortexes became necrotic within 7 days and glial cells accumulated at the boundary of the lesions within 14 days after the injury regardless of the cell types (or PBS) transplanted. In addition, in the injured cortexes of mice with WT/WT cell transplantation, 70 ± 3.0% of CMDil positive cells migrated across the boundary. We were interested in whether the mouse brains with WT/WT cell transplantation have reduced necrotic changes and reduced glial cell accumulation, leading to the size reduction of injured area of brains. However, we did not find any reproducible histopathological differences by HE staining in the injured areas between WT/WT NSPC transplantation groups and *yot/yot* NSPC transplantation groups except for existence of the grafted cells in the cortex. Further studies such as mouse MRI may disclose the differences after transplantation.

Seven days after initiation of cell cultures of *yot/yot* NSPCs (at the same time of use for transplantation), the expressions of Reelin associated receptors, the downstream signaling molecules, and motoneuron associated markers were only slightly reduced as compared with WT/WT NSPCs, except that the expressions of pDab1 and active Integrin β1 were almost completely inhibited in the *in vitro* culture (Table 1). In our preliminary 3-week *in vitro* cell culture experiments with differentiation inducing medium, *yot/yot* NSPCs exhibited lower viability and reduced differentiation capability into more mature neurons compared with WT/WT NSPCs (Suzuki et al., manuscript in preparation). It is possible that the mutated Dab1 gene of *yot/yot* mice rendered the NSPCs unresponsive to surrounding cues necessary to achieve cell survival and neural maturation. This may result in a decrease in motoneuron differentiation and possibly in viability of the mutant NSPCs in the striatum of injected WT/WT mouse brains.

We found that the mean survival time of the homozygous *yot/yot* mice was 22.0 ± 0.36 days (mean ± SEM of 26 mice), which was similar to that of reeler mice (Gallagher et al., 1998; Falconer, 1951). It is possible that neurons of the homozygous *yot/yot* mice have a limited life span both *in vitro* and *in vivo* (Cocito et al., 2016). It was reported that multipotent neurospheres from the embryonic forebrain of *yotari* mice tended to differentiate into astrocytes rather than neurons (Kwon et al., 2009). Further studies are needed to clarify these points.

Persistent expressions of pDab1 (Fig. 5E) and active Integrin α5β1 (Fig. 6C), both of which locate downstream of the Reelin signaling pathways may play a certain role in neural migration accompanying differentiation of WT/WT NSPCs toward cortical motoneuron phenotypes in the hemiplegic mouse brains.

It was suggested that pDab1 was sufficient to stimulate Srk, PI3K, Akt, and Rap1. Phosphorylated Dab1 regulated membrane protrusion, retraction, and ruffling in the absence of other signals (Wang and Cooper, 2017). Dab1 phosphorylation may be important for neural migration, leading to the functional recovery of the hemiplegic mice.

Cortical neurons are suggested to express specific transcriptional factors corresponding to the laminar position of the six-layered structure and the projected area. Ctip2 is thought to be a marker of subcerebral neurons in deep layer V (Molnár and Cheung, 2006). It is suggested that Crim1 is a marker of corticospinal motor neurons of layer V (Molnár and Cheung, 2006). Foxp2 is used as a marker of cortico-thalamic projection neurons in layer IV (Ferland et al., 2003). Neural progenitors expressed Fezf2 in the ventricular zone and later the expressions are observed in layer IV and V (Molyneaux et al., 2007).

In the injured cortex, Crim1 expressing cells increased from day 7 to day 14 (Fig. 7B). Ctip2 expressing cells and Fezf2 expressing cells

increased substantially from day 7 to day 28 in the injured cortex (Fig. 7*A,D*). It is likely that the NSPCs during and after migration interact with not only with individual grafted neurons by themselves but also with neighboring host neurons and their surrounding microenvironment. With these interactions, including those involving Reelin, NSPCs foster their differentiation toward motoneuron phenotypes and subsequent synapse formation.

It is reported that WT/WT NSPCs has heterogeneity such as the levels of Reelin, pDab1 protein and/or Integrin β1 protein expression (Alcántara et al., 1998; Förster et al., 2002; Howell et al., 1999). Therefore, it is possible that the heterogeneous WT/WT NSPCs responses against Reelin stimulation contributes to the variations of the motoneuron differentiation, their migration toward the damaged cortex and subsequent functional recovery of the mice.

Similarly, Ballout emphasized the importance in generation of corticofugal neuron subtypes, such as Ctip2 and Foxp2 expressing cells, from the mouse NSPCs after transplantation in adult lesioned brain (Ballout et al., 2016). The repression of neuron differentiation in the *yot/yot* NSPCs may relate to, somehow, the low Reelin expression by *yot/yot* NSPCs at the time of cell transplantation in this study (Fig. 1C, Table1).

We found that activation and/or modulation of Reelin mediated intracellular signaling pathway of neuronal cells may be beneficial for treating patients with brain damage and those with hemiplegia. Reelin-independent pathways were reported to be sufficient for inducing phosphorylation of Dab1 and subsequent neural migration (Andrade et al., 2007; Blake et al.,2008; Leeb et al., 2014; Zhang et al., 2018).

Phosphorylation of Dab1 is suggested to be important for neural migration, axon elongation and synapse regeneration. We are planning to select and characterize such kinases, in order to apply for patients with hemiplegia in near future.

In conclusion, Reelin and its downstream molecules play crucial roles in the neural regeneration and subsequent recovery of motor function in our hemiplegic mouse model.

Declaration of Competing Interest

None.

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References

Alcántara, S., Ruiz, M., D'Arcangelo, G., Ezan, F., de Lecea, L., Curran, T., Sotelo, C., Soriano, E., 1998. Regional and cellular patterns of reelin mRNA expression in the forebrain of the developing and adult mouse. J. Neurosci. 18, 7779–7799.

Andrade, N., Komnenovic, V., Blake, S.M., Jossin, Y., Howell, B., Goffinet, A., Schneider, W.J., Nimpf, J., 2007. ApoER2/VLDL receptor and Dab1 in the rostral migratory stream function in postnatal neuronal migration independently of Reelin. Proc. Natl. Acad. Sci. U. S. A. 104, 8508–8513.

Arimitsu, N., Shimizu, J., Fujiwara, N., Takai, K., Takada, E., Kono, T., Ueda, Y., Suzuki, T., Suzuki, N., 2012. Role of SDF1/CXCR4 interaction in experimental hemiplegic models with neural cell transplantation. Int. J. Mol. Sci. 13, 2636–2649. https://doi.org/10.3390/ijms13032636.

Arnaud, L., Ballif, B.A., Forster, E., Cooper, J.A., 2003. Fyn tyrosine kinase is a critical regulator of disabled-1 during brain development. Curr. Biol. 13, 9–17.

Ballif, B.A., Arnaud, L., Arthur, W.T., Guris, D., Imamoto, A., Cooper, J.A., 2004. Activation of a Dab1/CrkL/C3G/Rap1 pathway in Reelin-stimulated neurons. Curr. Biol. 14, 606–610.

Ballout, N., Frappé, I., Péron, S., Jaber, M., Zibara, K., Gaillard, A., 2016. Development and maturation of embryonic cortical neurons grafted into the damaged adult motor cortex. Front. Neural. Circuits 10, 55. https://doi.org/10.3389/fncir.2016.00055.

Bock, H.H., Herz, J., 2003. Reelin activates SRC family tyrosine kinases in neurons. Curr. Biol. 13, 18–26.

Brai, E., Marathe, S., Astori, S., Fredj, N.B., Perry, E., Lamy, C., Scotti, A., Alberi, L., 2015. Notch1 regulates hippocampal plasticity through interaction with the Reelin pathway, glutamatergic transmission and CREB signaling. Front. Cell. Neurosci. 9, 447. https://doi.org/10.3389/fncel.2015.00447.

Chiba, S., Iwasaki, Y., Sekino, H., Suzuki, N., 2003. Transplantation of motoneuron-enriched neural cells derived from mouse embryonic stem cells improves motor function of hemiplegic mice. Cell Transplant. 12, 457–468.

Chiba, S., Ikeda, R., Kurokawa, M.S., Yoshikawa, H., Takeno, M., Nagafuchi, H., Tadokoro, M., Sekino, H., Hashimoto, T., Suzuki, N., 2004. Anatomical and functional recovery by embryonic stem cell-derived neural tissue of a mouse model of brain damage. J. Neurol. Sci. 219, 107–117.

Cocito, C., Merighi, A., Giacobini, M., Lossi, L., 2016. Alterations of cell proliferation and apoptosis in the hypoplastic reeler cerebellum. Front. Cell. Neurosci. 10, 141. https://doi.org/10.3389/fncel.2016.00141.

D'Arcangelo, G., Curran, T., 1998. Reeler: new tales on an old mutant mouse. Bioessays 20, 235–244.

Falconer, D.S., 1951. Two new mutants, 'trembler' and 'reeler', with neurological actions in the house mouse (Mus musculus L.). J. Genet. 50, 192–201.

Fatemi, S.H., Earle, J.A., McMenomy, T., 2000. Reduction in Reelin immunoreactivity in hippocampus of subjects with schizophrenia, bipolar disorder and major depression. Mol. Psychiatry 5, 654–663, 571.

Fatemi, S.H., Snow, A.V., Stary, J.M., Araghi-Niknam, M., Reutiman, T.J., Lee, S., Brooks, A.I., Pearce, D.A., 2005. Reelin signaling is impaired in autism. Biol. Psychiatry 57, 777–787.

Ferland, R.J., Cherry, T.J., Preware, P.O., Morrisey, E.E., Walsh, C.A., 2003. Characterization of Foxp2 and Foxp1 mRNA and protein in the developing and mature brain. J. Comp. Neurol. 460, 266–279.

Förster, E., Tielsch, A., Saum, B., Weiss, K.H., Johanssen, C., Graus-Porta, D., Müller, U., Frotscher, M., 2002. Reelin, disabled 1, and beta 1 integrins are required for the formation of the radial glial scaffold in the hippocampus. Proc. Natl. Acad. Sci. U. S. A. 99, 13178–13183.

Franco, S.J., Martinez-Garay, I., Gil-Sanz, C., Harkins-Perry, S.R., Müller, U., 2011. Reelin regulates cadherin function via Dab1/Rap1 to control neuronal migration and lamination in the neocortex. Neuron 69, 482–497. https://doi.org/10.1016/j.neuron.2011.01.003.

Gallagher, E., Howell, B.W., Soriano, P., Cooper, J.A., Hawkes, R., 1998. Cerebellar abnormalities in the disabled (mdab1-1) mouse. J. Comp. Neurol. 402, 238–251.

Hazama, Y., Kurokawa, M.S., Chiba, S., Tadokoro, M., Imai, T., Kondo, Y., Nakatsuji, N., Suzuki, T., Hashimoto, T., Suzuki, N., 2010. SDF1/CXCR4 contributes to neural regeneration in hemiplegic mice with a monkey ES-cell-derived neural graft. Inflamm. Regen. 30, 193–205.

Hong, S.E., Shugart, Y.Y., Huang, D.T., Shahwan, S.A., Grant, P.E., Hourihane, J.O., Martin, N.D., Walsh, C.A., 2000. Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. Nat. Genet. 26, 93–96.

Howell, B.W., Herrick, T.M., Hildebrand, J.D., Cooper, J.A., 1999. Reelin-induced tyrosine phosphorylation of disabled 1 during neuronal positioning. Genes Dev. 13, 643–648.

Howell, B.W., Herrick, T.M., Hildebrand, J.D., Zhang, Y., Cooper, J.A., 2000. Dab1 tyrosine phosphorylation sites relay positional signals during mouse brain development. Curr. Biol. 10, 877–885.

Ikeda, R., Kurokawa, M.S., Chiba, S., Yoshikawa, H., Hashimoto, T., Tadokoro, M., Suzuki, N., 2004. Transplantation of motoneurons derived from MASH1-transfected mouse ES cells reconstitutes neural networks and improves motor function in hemiplegic mice. Exp. Neurol. 189, 280–292.

Ikeda, R., Kurokawa, M.S., Chiba, S., Yoshikawa, H., Ide, M., Tadokoro, M., Nito, S., Nakatsuji, N., Kondoh, Y., Nagata, K., Hashimoto, T., Suzuki, N., 2005. Transplantation of neural cells derived from retinoic acid-treated cynomolgus monkey embryonic stem cells successfully improved motor function of hemiplegic mice with experimental brain injury. Neurobiol. Dis. 20, 38–48.

Imai, H., Shoji, H., Ogata, M., Kagawa, Y., Owada, Y., Miyakawa, T., Sakimura, K., Terashima, T., Katsuyama, Y., 2017. Dorsal forebrain-specific deficiency of Reelin-Dab1 signal causes behavioral abnormalities related to psychiatric disorders. Cereb. Cortex 27, 3485–3501. https://doi.org/10.1093/cercor/bhv334.

Ishii, K., Kubo, K.I., Nakajima, K., 2016. Reelin and neuropsychiatric disorders. Front. Cell. Neurosci. 10, 229.

Jossin, Y., Cooper, J.A., 2011. Reelin, Rap1 and N-cadherin orient the migration of multipolar neurons in the developing neocortex. Nat. Neurosci. 14, 697–703.

Katyal, S., Godbout, R., 2004. Alternative splicing modulates Disabled-1 (Dab1) function in the developing chick retina. EMBO J. 23, 1878–1888.

Kojima, T., Nakajima, K., Mikoshiba, K., 2000. The disabled 1 gene is disrupted by a replacement with L1 fragment in yotari mice. Mol. Brain Res. 75, 121–127.

Kono, T., Arimitsu, N., Shimizu, J., Takai, K., Fujiwara, N., Umehara, T., Saito, A., Ueda, Y., Wakisaka, S., Suzuki, T., Hashimoto, T., Tanaka, Y., Suzuki, N., 2013. Human iPS cell derived neurons with cortical motor neuron phenotype are relevant for functional recovery of hemiplegic mice with injured motor cortex. St. Marianna Med. J. 4, 31–40.

Kwon, I.S., Cho, S.K., Kim, M.J., Tsai, M.J., Mitsuda, N., Suh-Kim, H., Lee, Y.D., 2009. Expression of disabled 1 suppresses astroglial differentiation in neural stem cells. Mol. Cell. Neurosci. 40, 50–61. https://doi.org/10.1016/j.mcn.2008.08.012.

Leeb, C., Eresheim, C., Nimpf, J., 2014. Clusterin is a ligand for apolipoprotein E receptor 2 (ApoER2) and very low density lipoprotein receptor (VLDLR) and signals via the Reelin-signaling pathway. J. Biol. Chem. 289, 4161–4172. https://doi.org/10.1074/jbc.M113.529271.

Matsubayashi, Y., Iwai, L., Kawasaki, H., 2008. Fluorescent double-labeling with carbocyanine neuronal tracing and immunohistochemistry using a cholesterol-specific detergent digitonin. J. Neurosci. Methods 174, 71–81.

Molnár, Z., Cheung, A.F., 2006. Towards the classification of subpopulations of layer V pyramidal projection neurons. Neurosci. Res. 55, 105–115.

Molyneaux, B.J., Arlotta, P., Menezes, J.R., Macklis, J.D., 2007. Neuronal subtype specification in the cerebral cortex. Nat. Rev. Neurosci. 8, 427–437.

Pacey, L.K.K., Stead, S., Gleave, J.A., Tomczyk, K., Doering, L.C., 2006. Neural stem cell culture: Neurosphere generation, microscopical analysis and cryopreservation. Nat. Protoc. Exch. https://doi.org/10.1038/nprot.2006.215.

Park, T.J., Curran, T., 2008. Crk and Crk-like play essential overlapping roles downstream of disabled-1 in the Reelin pathway. J. Neurosci. 28, 13551–13562. https://doi.org/10.1523/JNEUROSCI.4323-08.2008.

Pramatarova, A., Ochalski, P.G., Chen, K., Gropman, A., Myers, S., Min, K.T., Howell, B.W., 2003. Nck beta interacts with tyrosine-phosphorylated disabled 1 and redistributes in Reelin-stimulated neurons. Mol. Cell. Biol. 23, 7210–7221.

Pujadas, L., Gruart, A., Bosch, C., Delgado, L., Teixeira, C.M., Rossi, D., de Lecea, L., Martínez, A., Delgado-García, J.M., Soriano, E., 2010. Reelin regulates postnatal neurogenesis and enhances spine hypertrophy and long-term potentiation. J. Neurosci. 30, 4636–4649. https://doi.org/10.1523/JNEUROSCI.5284-09.2010.

Pujadas, L., Rossi, D., Andrés, R., Teixeira, C.M., Serra-Vidal, B., Parcerisas, A., Maldonado, R., Giralt, E., Carulla, N., Soriano, E., 2014. Reelin delays amyloid-beta fibril formation and rescues cognitive deficits in a model of Alzheimer's disease. Nat. Commun. 5 (3443). https://doi.org/10.1038/ncomms4443.

Sekine, K., Kawauchi, T., Kubo, K., Honda, T., Herz, J., Hattori, M., Kinashi, T., Nakajima, K., 2012. Reelin controls neuronal positioning by promoting cell-matrix adhesion via inside-out activation of integrin α5β1. Neuron 76, 353–369. https://doi.org/10.1016/j.neuron.2012.07.020.

Suzuki, N., Arimitsu, N., Shimizu, J., Takai, K., Hirotsu, C., Ueda, Y., Wakisaka, S., Fujiwara, N., Suzuki, T., 2017. Neuronal cell sheets of cortical motor neuron phenotype derived from human iPSCs. Cell Transplant. 26, 1355–1364. https://doi.org/10.1177/0963689717720280.

Trotter, J., Lee, G.H., Kazdoba, T.M., Crowell, B., Domogauer, J., Mahoney, H.M., Franco, S.J., Müller, U., Weeber, E.J., D'Arcangelo, G., 2013. Dab1 is required for synaptic plasticity and associative learning. J. Neurosci. 33, 15652–15668. https://doi.org/10.1523/JNEUROSCI.2010-13.2013.

Wang, L., Cooper, J.A., 2017. Optogenetic control of the Dab1 signaling pathway. Sci. Rep. 7, 43760. https://doi.org/10.1038/srep43760.

Yoneshima, H., Nagata, E., Matsumoto, M., Yamada, M., Nakajima, K., Miyata, T., Ogawa, M., Mikoshiba, K., 1997. A novel neurological mutant mouse, yotari, which exhibits reeler-like phenotype but expresses CR-50 antigen/reelin. Neurosci. Res. 29, 217–223.

Zhang, J.H., Zhao, Y.F., He, X.X., Zhao, Y., He, Z.X., Zhang, L., Huang, Y., Wang, Y.B., Hu, L., Liu, L., Yu, H.L., Xu, J.H., Lai, M.M., Zhao, D.D., Cui, L., Guo, W.X., Xiong, W.C., Ding, Y.Q., Zhu, X.J., 2018. DCC-mediated Dab1 phosphorylation participates in the multipolar-to-bipolar transition of migrating neurons. Cell Rep. 22, 3598–3611. https://doi.org/10.1016/j.celrep.2018.03.005.

Zhao, Y.H., Lam, D., Yang, J., Lin, J., Tham, C.K., Ng, W.H., Wang, S., 2012. Targeted suicide gene therapy for glioma using human embryonic stem cell-derived neural stem cells genetically modified by baculoviral vectors. Gene Ther. 19, 189–200.