Complete Loss of Ndel1 Results in Neuronal Migration Defects and Early Embryonic Lethality

Shinji Sasaki,1† Daisuke Morii,2 Kazuhiro Toyo-oka,2 Amy Chen,3 Lisa Garrett-Beal,3 Masami Muramatsu,1 Shuji Miyagawa,4 Noriko Hiraizawa,5 Atushi Yoshikii,5 Anthony Wynshaw-Boris,6 and Shinji Hirotsune1,2,*

Division of Neuro-Science, Research Center for Genomic Medicine, Saitama Medical School, Yamane 1397-1, Hitada City, Saitama 350-1241, Japan; Department of Genetic Disease Research, Osaka City University Graduate School of Medicine, Asahi-machi 1-4-3, Abeno, Osaka 545-8586, Japan; Genetic Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Building 49, Room 4C80, 49 Convent Dr., Bethesda, Maryland 20892; Division of Organ Transplantation, Biomedical Research Center, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan; Experimental Animal Division, Department of Biological Systems, BioResource Center, RIKEN Tsukuba Institute, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan; and Departments of Pediatrics and Medicine, UCSD Cancer Center, University of California, San Diego School of Medicine, 9500 Gilman Dr., Mailstop 0627, La Jolla, California 92093-0627.

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Regulation of cytoplasmic dynein and microtubule dynamics is crucial for both mitotic cell division and neuronal migration. NDEL1 was identified as a protein interacting with LIS1, the protein product of a gene mutated in the lissencephaly. To elucidate NDEL1 function in vivo, we generated null and hypomorphic alleles of Ndel1 in mice by targeted gene disruption. Ndel1+/− mice were embryonic lethal at the peri-implantation stage like null mutants of Lis1 and cytoplasmic dynein heavy chain. In addition, Ndel1−/− blastocysts failed to grow in culture and exhibited a cell proliferation defect in inner cell mass. Although Ndel1+/− mice displayed no obvious phenotypes, further reduction of NDEL1 by making null/hypomorphic compound heterozygotes (Ndel1+/−/+−) resulted in histological defects consistent with mild neuronal migration defects. Double Lis1−/−/− mice displayed more severe neuronal migration defects than Lis1−/−/+−. Ndel1+/− mice were embryonic lethal at the peri-implantation stage like null mutants of Lis1 and cytoplasmic dynein heavy chain. In addition, Ndel1−/− blastocysts failed to grow in culture and exhibited a cell proliferation defect in inner cell mass. Although Ndel1+/− mice displayed no obvious phenotypes, further reduction of NDEL1 by making null/hypomorphic compound heterozygotes (Ndel1+/−/+−) resulted in histological defects consistent with mild neuronal migration defects. Double Lis1−/−/− mice displayed more severe neuronal migration defects than Lis1−/−/+−.

The mammalian brain is assembled through a series of far-ranging migrations that result in the segregation of neurons with similar properties into discrete layers (32). Important clues for molecular mechanisms of neuronal migration were provided from the analysis of brain malformations in humans ranging migrations that result in the segregation of neurons with similar properties into discrete layers (32). Important clues for molecular mechanisms of neuronal migration were provided from the analysis of brain malformations in humans

* Corresponding author. Mailing address: Department of Genetic Disease Research, Osaka City University Graduate School of Medicine, Asahi-machi 1-4-3 Abeno, Osaka 545-8586, Japan. Phone: 6-6645-3725. Fax: 6-6645-3727. E-mail: shinjii@med.osaka-cu.ac.jp.
† Present address: Department of Anatomy, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.
FIG. 1. Generation of a gene-disrupted mouse of Ndel1 [Ndel1cko(III): Ndel1cko(III)]. (A) Summary of targeting strategy for the Ndel1 locus. A diagrammatic representation of the targeting vector and genomic loci of Ndel1 gene is shown. Exons are represented by gray boxes. In this gene targeting, Ndel1 function was expected to be intact (Ndel1cko(III)). Exon 3 was removed by CRE-mediated recombination to inactivate Ndel1 function [Ndel1cko(III)]. (B) Southern blot analysis of tail DNA from wild-type (+/-, lane 1) and heterozygous mutant (+/-, lanes 2 and 3) animals. (C) Southern blot analysis of tail DNA from deletion mutant (+/del, lane 1), wild-type (+/+, lane 2), and heterozygous mutant (+/loxP, lane 3) mice. CRE-mediated deletion of exon 3 was evaluated by Southern blotting. Note the difference in band sizes after deletion. We used Ndel1cko(III) as a Ndel1+/- in the all experiment.
FIG. 2. Generation of another gene disrupted mouse of Ndel1[Ndel1\textsuperscript{cko(IV)}]. (A) Summary of the second targeting strategy for the Ndel1 locus. A diagrammatic representation of the targeting vector and genomic loci of Ndel1 gene is shown. PGK-neo was inserted into intron IV, which was larger than intron III. Exon IV was removed by CRE-mediated recombination. (B) Southern blot analysis of ES cell DNA. By digestion with Bst1107I, the wild-type allele, the targeted allele that lost the loxP site, and the targeted allele that preserved the loxP site were discriminated (arrows). (C) Using this conditional KO line, we were able to make conditional homozygotes. Primers are shown on the right side (arrows). (D) CRE expression efficiently removed the DNA fragment that was demarcated by loxP sequence. (E) We mated new conditional KO mice and KO mice to make compound heterozygotes. Primers are shown on the right side. Compound heterozygotes were completely viable and fertile. We used Ndel1\textsuperscript{cko(IV)} as Ndel1\textsuperscript{cko/−} in all experiments.
two mammalian homologues of Aspergillus NudE. NDE1 (8) and NDE1L (29, 35, 36); the latter, a homologue of NudE from Aspergillus nidulans, is a LIS1 binding protein that participates with LIS1 in the regulation of CDC42 function via phosphorylation by CDK5/p35 (26, 27, 29, 35, 36). We have also shown that phosphorylation of NDE1L is protected by the serine-threonine binding protein 14-3-3ε and that 14-3-3ε is required for normal neuronal migration (42), providing further support that the phosphorylation of NDE1L is required for proper neuronal migration. NDE1 has a similar function in cells but is expressed later in development than NDE1L. Recently, Ndel1-null mice have been produced (9). These mice are viable and display microcephaly. They display defects in neurogenesis that result from reduced progenitor cell division and progenitor cell fate, as well as moderate defects in neuronal migration. To date, targeted mutants of Ndel1 have not been generated, and the in vitro phenotype of null mutants of Ndel1 is unknown.

To understand the role of NDE1L in vivo and to determine whether it plays a role in corticogenesis and cell division, we generated Ndel1-disrupted mice. We found that, similar to Lis1, Ndel1 mutant mice display a dosage-dependent neuronal migration phenotype, and complete loss of Ndel1 resulted in perinatal lethality. Examination of in vivo and in vitro phenotypes suggests that NDE1L, LIS1, and NDE1 act in a common pathway to regulate dynein, but each has distinct roles in the regulation of microtubule organization and neuronal migration.

### MATERIALS AND METHODS

**Generation and analysis of Ndel1 KO mice.** To understand the function of Ndel1 in vivo, we generated a first conditional knockout (KO) mouse to inactivate Ndel1 by Cre-mediated recombination. We assembled a targeting construct in which a PGK-neo gene flanked by a loxP site and an additional loxP site were inserted into intron II and intron III, respectively. The linearized targeting construct was introduced into TC1 embryonic stem (ES) cells (2) from a 129S6 background by electroporation. The targeted ES clones were screened by Southern blot and injected into blastocysts to create chimeric mice. Highly agouti chimeric males were mated to wild-type females to give rise to heterozygotes for the conditional allele [Ndel1<sup>flk(III)</sup>/+], which was identified by Southern blot analysis and PCR. These heterozygous mice were mated with Ella-<sup>Cre</sup> germ line deleter transgenic mice (19). Offspring from the matings between an Ndel1<sup>flk(III)/+</sup> line and an Ella-<sup>Cre</sup> transgene line were genotyped by Southern blot analysis and PCR. Southern blot analysis and PCR examination indicated efficient deletion of the fragment carrying exon III by Cre mediated recombinocination in vivo. Ndel1<sup>flk(III)/+</sup>/EIIa-Cre mice exhibited embryonic lethality similar with Ndel1<sup>flk(III)/+</sup>/EIIa-Cre mice, suggesting that the presence of the neo gene severely affects expression of Ndel1. It was not possible to remove neo gene by itself. We generated a first conditional knockout (KO) mouse line and an Ella-<sup>Cre</sup> transgene line were genotyped by Southern blot analysis and PCR. Southern blot analysis and PCR examination indicated efficient deletion of the fragment carrying exon III by Cre-mediated recombinocination in vivo. Ndel1<sup>flk(III)/+</sup>/EIIa-Cre mice exhibited embryonic lethality similar with Ndel1<sup>flk(III)/+</sup>/EIIa-Cre mice, suggesting that the presence of the neo gene severely affects expression of Ndel1. It was not possible to remove neo gene by itself. For the second conditional KO strategy, we inserted a PGK-neo gene flanked by a loxP site and an additional loxP site into intron IV and intron III, respectively. Highly agouti chimeric mice were mated to wild-type females to give rise to heterozygotes for the second conditional allele [Ndel1<sup>flk(IV)/+</sup>]. In contrast to the first line of conditional KO, heterozygotes of the second conditional KO [Ndel1<sup>flk(IV)/+</sup>/EIIa-Cre] line was completely viable and fertile. CRE-mediated deletion of the second conditional KO mouse line was also confirmed by PCR with primers located flanking the site of the deleted region. We used Ndel1<sup>flk(IV)/+</sup>/EIIa-Cre, whereas Ndel1<sup>flk(IV)/+</sup>/EIIa-Cre was used as Ndel1<sup>flk(IV)/+</sup> in this experiment. Expression of Ndel1 was examined by Western blotting with the C-6 NDE1L specific antibody (36) and Northern blotting with a full-length Ndel1 cDNA fragment. All experiments with mouse models were performed based on the animal experiment guidelines of our universities.

**Histological examination and immunohistochemistry.** After perfusion with Bouin’s or 4% paraformaldehyde fixative, tissues from wild-type and various mutant mice were subsequently embedded in paraffin and sectioned at 5-μm thickness. After deparaffinization, endogenous peroxidase activity was blocked by immersion of the sections in 1% H<sub>2</sub>O<sub>2</sub> for 30 min. The sections were then boiled in 0.01 M citrate buffer (pH 6.0)/liter for 20 min and cooled slowly. Before staining, the sections were blocked with rodent block (LabVision) for 60 min. The sections were washed in phosphate-buffered saline and incubated with each antibody. Primary cultures of cerebellar granule cells were generated from 5-day-old C57Bl/6 pups as described previously (36). Cells were plated at a density of 2.5 × 10<sup>3</sup> cells/cm<sup>2</sup> and maintained in basic Eagle medium containing 10% fetal calf serum, 25 mM KCl, 2 mM glutamine, and 50 mg of gentamicin/mL. After 18 to 20 h, cytosome arabinose (10 mM) was added to the culture media to halt non-neuronal cell proliferation. Blastocysts were collected by flushing the oviducts of female mice at 3.5 days postcoitum and cultured individually for 3 days on gelatinized coverslips in ES cell medium. Cultured blastocysts were fixed in 2% paraformaldehyde–phosphate-buffered saline for 10 min at room temperature. Some samples were fixed in 2% paraformaldehyde and 0.2% glutaraldehyde for 10 min at room temperature and blocked in 1 mg of NaBH<sub>4</sub>/ml for 30 min at 4°C. After primary culture from each tissue, cells were fixed in cold methanol (−20°C) and postfixed by 3% formaldehyde. Incubation with 0.15% Triton X-100 was used for cell permeabilization after fixation. Immunohistochemistry was performed based on the standard procedure. Apoptotic cells were detected by TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling) assay (Chemicon). Incorporated biotin was detected with an ABC kit and visualized with diaminobenzidine.

**Primary cell culture, transfection, and immunofluorescence.** Establishment of mouse embryonic fibroblasts (MEFs) was performed as previously described (17, 36). A red fluorescent protein (RFP)-tagged Cre expression vector was introduced into these primary culture cells by using Lipofectamine 2000 reagents (Invitrogen). After CRE-mediated inactivation of Lis1 or Ndel1 gene, these cells were subjected to immunohistochemistry by using anti-β-tubulin antibody (Clone TUB 2.1; Sigma) or anti-β-COP antibody (Sigma). For rescue experiments, green fluorescent protein (GFP)-conjugated to Lis1, NDE1L, or NDE1 was simultaneously transfected with the CRE expression vector.

**Reaggregate neuronal migration assay.** Cerebellar granule neurons were dissociated from postnatal 5-day-old mice (17, 41) and transfected with various vectors by using Lipofectin (LipoTrust SR reagents in 100 ml. After 18 to 20 h, cytosine arabinoside (10 mM) was added to the culture media to halt non-neuronal cell proliferation. Blastocysts were collected by flushing the oviducts of female mice at 3.5 days postcoitum and cultured individually for 3 days on gelatinized coverslips in ES cell medium. Cultured blastocysts were fixed in 2% paraformaldehyde–phosphate-buffered saline for 10 min at room temperature. Some samples were fixed in 2% paraformaldehyde and 0.2% glutaraldehyde for 10 min at room temperature and blocked in 1 mg of NaBH<sub>4</sub>/ml for 30 min at 4°C. After primary culture from each tissue, cells were fixed in cold methanol (−20°C) and postfixed by 3% formaldehyde. Incubation with 0.15% Triton X-100 was used for cell permeabilization after fixation. Immunohistochemistry was performed based on the standard procedure. Apoptotic cells were detected by TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling) assay (Chemicon). Incorporated biotin was detected with an ABC kit and visualized with diaminobenzidine.

**RESULTS OF COMPLETE LOSS OF NDEL1.**

<table>
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<th>Embryo (days p.c.) or mouse</th>
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* a/− = agouti chimeras were mated within 12 h of birth. Weaning mice were typically 4 weeks of age. p.c., postcoitum.

* The number of embryos includes the number resorbed.

* Genotypes for embryos were determined by PCR analysis of tissue lysate of blastocyst outgrowth for E3.5 and of yolk sac for E7.5 to E13.5. Genotypes for newborn and weaning mice were determined by PCR analysis of tail DNA.

**TABLE 1. Embryos and mice used in this study**
FIG. 3. Embryonic lethality of Ndel1-null mutants. (A) Homozygous mutants of Ndel1 were not observed, suggesting that null mutants are embryonic lethal. Normal (left) and degenerated decidua (right) resulting from the Ndel1<sup>−/−</sup> cross were sectioned at E5.5 and stained with hematoxylin and eosin. ec, ectoplacental cone; ee, extra-embryonic ectoderm; e, epiblast. (B) Wild-type (upper) and homozygous (lower) blastocysts were isolated from a heterozygous cross and cultured. Both blastocysts hatched and differentiated into trophoblast (tr) and inner cell mass (ICM). The inner cell mass of homozygotes exhibited poor growth and degenerated. (C) Quantitation of the amount of Ndel1 expression in the various genotypes. Northern blot (left side) and Western blot (right side) are shown. Expression was calculated by densitometry compared to the wild type. We repeated three independent experiments, and the results were highly reproducible. (D) Expression of LIS1 or NDEL1 in Ndel1 KO mice or Lis1 KO mice was examined. Total protein was extracted from a brain of E15.5 embryo and subjected to Western blotting. There was no obvious difference in LIS1 or NDEL1 expression in Ndel1 or Lis1 KO mice, respectively.
RESULTS

Targeted gene disruption of Ndel1. To explore the in vivo role of NDEL1 and genetic interaction between Ndel1 and Lis1 during mitotic cell division and neuronal development, we generated two independent Ndel1 conditional KO lines (Fig. 1 and Fig. 2; see also Materials and Methods). We could not delete the PGK-neo gene used for selection in ES cells in the exon III floxed allele, and the undeleted allele with the neo insertion had a homozygous lethal phenotype identical to that of the null allele produced after deletion of the PGK-neo gene and exon III (see Materials and Methods). Therefore, we produced a conditional allele that floxed exon IV. We used mice in which exon III of Ndel1 was deleted as Ndel1+/H11001/H11002, whereas we used mice in which exon IV of Ndel1 was conditionally deleted as Ndel1cko/H11001.

Mice heterozygous for the Ndel1 gene (Ndel1+/−) were outwardly normal, fertile born in appropriate Mendelian ratios. Ndel1+/− mice were bred to produce homozygous mutants (Ndel1−/−). An analysis of F2s from the mating of Ndel1+/− mice showed that the genotype ratios of +/− to +/− to −/− animals were 70:154:0, suggesting that the complete loss of Ndel1 resulted in embryonic lethality (Table 1). Therefore, we examined embryos from heterozygous crosses at several developmental stages. Cumulative genotyping between E7.5 and E13.5 demonstrated that the ratios of +/− to +/− to −/− mice were 26:54:0 (Table 1). We dissected embryos at between E5.5 and E13.5 and found degenerated embryos and empty deciduas only at the earliest postimplantation stages (Fig. 3A). We found 35 normal embryos (80%) and 10 empty decidua (20%) at E5.5 among 45 animals, and there were 5 +/− (21%), 15 +/− (66%), and 3 −/− (13%) blastocyst cultures at E3.5 among 23 animals. These findings suggested that Ndel1−/− mice are lethal at very early stages of embryogenesis. This is in contrast to Nde1-null mice, which are viable and display microcephaly from defects in neurogenesis (9).

To define the role of NDEL1 in the early embryo, we examined the behavior of blastocyst explants isolated from (Sigma-Aldrich)-treated slides. After 15 h, the distance between cell bodies and the edge of reaggregates was measured. The details of the experimental procedure have been described (17, 41). An RFP-tagged Cre expression vector with or without GFP-tagged LIS1, NDEL1, or NDE1 vector were transfected as described above.

FIG. 4. Synergistic effects of Lis1 and Ndel1 mutations on brain morphogenesis. Genotypes are shown at the top of the panels. (A) Midsagittal sections of cerebral cortex (upper) and hippocampus (lower). Lis1cko+/− or Ndel1−/− samples were grossly normal. In contrast, mild cell dispersion of CA3 region was observed in Ndel1−/− compound heterozygotes or Lis1cko+ and Ndel1−/− double heterozygotes (arrowheads). (B) Loss of cell compaction (arrowheads) in CA3 region was clearly observed by NeuN staining. (C) Abnormal corticogenesis was characterized by fragmentation of the subplate layer (arrowheads) which was visualized by MAP2 staining (upper panels) and chondroitin sulfate proteoglycan staining (lower panels) at E15.5. MZ, marginal zone; CP, cortical plate; SP, subplate; IZ, intermediate zone.
FIG. 5. Examination of migration defects of *Lis1* and *Ndell* mutants. (A) BrdU birth dating analysis reveals neuronal migration defects in the cross of *Ndell*<sup>−/−</sup> and *Ndell*<sup>cko/−</sup>, in the cross of *Lis1*<sup>−/−</sup> and *Ndell*<sup>−/−</sup>, or in the cross of *Lis1*<sup>−/−</sup> and *Ndell*<sup>−/−</sup>. Genotypes are described at the top of the panels. Mice were injected with BrdU at E15.5 and sacrificed at P0. Quantitative analysis was performed by measuring the distribution of BrdU-labeled cells in each bin which equally divided the cortex from the molecular layer (ML) to the subplate (SP). The staining patterns are representative of three different experiments. Note the shift downward toward the ventricular side as *Lis1* or *Ndell* dosage was reduced. CPs, cortical plate surface; CPI, cortical plate inner. (B) Summary of mean migration distance of each genotype.
FIG. 6. Specific defect of corticogenesis and apoptotic cell death by Lis1 or Ndel1 disruption. (A) Quantitation of cell number of the cortex in various combination of Lis1 KO and/or Ndel1 KO mice. After hematoxylin and eosin staining of the sagittal sections of the cortices of 8W adult mice, samples were subjected to cell counting. The cell counts of three corresponding sections in each genotype were determined. The histogram indicates the cell number. Mild reduction of cell number was observed in Lis1+/−, Ndel1+/−, and Lis1+/−/Ndel1−/− mice. (B) Cortical phenotypes of Lis1 and/or Ndel1 mutants (adult, 8W) were examined by layer specific makers, calbindin (layer 2 and 3) and c-Neu (layer 5). We measured five independent sides from each genotype. Total number of calbindin-positive cells is shown at the bottom of each panel. Calbindin-positive cells were more dispersed in Ndel1−/−/Lis1−/−, Ndel1+/−/Lis1−/−, Lis1−/−/Lis1−/−, and Lis1−/−/Ndel1−/− mice, whereas c-Neu-positive cells were more broadly distributed in Ndel1−/−/Lis1−/−, Ndel1+/−/Lis1−/−, Lis1−/−/Lis1−/−, and Ndel1−/−/Lis1−/− mice. Although the distribution became more broad and overlapped, inversion of layering was not observed. Genotypes are noted at the top of the panel. (C) Histogram plots of the relative frequency of calbindin-positive cells and c-Neu-positive cells to the total cell number in each genotype. Notably, later-migrating neurons (calbindin-positive cells) were more sensitive to LIS1 and NDEL1 dosage. (D) Apoptotic cell death was examined by TUNEL stainong at E11.5 and E15.5. We measured five independent sides from each genotype. The total number of calbindin-positive cells was examined by TUNEL staining at E11.5 and E15.5. We measured five independent sides from each genotype. The total number of calbindin-positive cells is shown at the bottom of each panel. (E) Histogram plot of the relative frequency of TUNEL-positive cell to the total number of cells in each genotype. There was no obvious increase of cell death in early generated neurons. In contrast, significant enhancement of cell death was observed in neurons generated later, a finding consistent with loss of calbindin-positive cells, and cell death was highly correlated with the reduction of LIS1 dosage.
RESULTS OF COMPLETE LOSS OF Ndel1

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Ndel1+/− crosses in culture. Genotypes of explants were performed by PCR at the end of the experiment. Wild-type, heterozygous (data not shown), and homozygous blastocysts attached to the substratum. Trophoblast cells from wild-type blastocyst explants flattened and expanded, while the inner cell mass grew on the top of the trophoblast cells (Fig. 3B). In contrast, the inner cell mass cells of Ndel1+/− explants grew poorly after 3 days and degenerated soon thereafter. These phenotypes were previously observed in Lis1 or CDHC KO mice (14, 17).

We quantitated Ndel1 mRNA and protein expression by Northern (Fig. 3C, left) blotting and Western blotting (Fig. 3C, right), respectively. Insertion of the neo gene into intron IV partially suppressed Ndel1 expression (30%), demonstrating that the conditional allele which carries the neo gene acts as a hypomorphic mutant. To test whether either Lis1 or Ndel1 reactivation in Ndel1−/−MEFs, but GFP-LIS1 and GFP-NDE1 were less effective (see panel B). To address dynein regulation by LIS1 and NDEL1, we examined the distribution of Lis1−/−MEFs (C) and in Ndel1−/−MEFs generated by CRE-mediated recombination. Cotransfected plasmids for rescue were shown on the top. (A and B) The loss of LIS1 causes a redistribution and an enrichment of microtubules near the nucleus (A), whereas loss of NDEL1 results in amorphous microtubules (B). Perinuclear accumulation of microtubules in Lis1−/−/H9252 mice (C) and in Ndel1−/−/H11001 mice, (Fig. 4A). Although Lis1−/−/H11001 mice displayed migration defects associated with the reduction of NDEL1, the reduction of NDEL1 became more severe in the presence of Lis1−/−/H11001 mice (5A), Ndel1+/−/H9252−/−Lis1−/−/H11001−/− mutants displayed migration defects, additional heterozygous loss of NDEL1 significantly reduced the cell population that reached layer II (Fig. 5A, Ndel1+/−/H9252−/−Lis1−/−/H11001−/−). Although heterozygous loss of NDEL1 was not sufficient for the creation of a clear migration defect as in the Lis1−/−/H11001−/− mutants, combinatorial loss of Lis1−/−/H11001−/− and Lis1−/−/H11001−/− clearly led to a migration defect (Fig. 3B), suggesting that Lis1 and NDEL1 cooperatively function during neuronal migration in the cortex, as well as in the hippocampus.

**Neuronal migration defect in Ndel1 disrupted mice and genetic interaction with Lis1.** In the adult brain (8W), Ndel1+/− mice displayed no obvious migration defects in either the neocortex or hippocampus (Fig. 4A). We further reduced the level of NDEL1 expression to ca. 20% of wild-type by making compound heterozygotes (Ndel1+/−/H9252). Ndel1+/−/H11001−/− mice exhibited mild partial splitting and diffuse pyramidal cells in the CA3 and CA2 region of the hippocampus (Fig. 4A). To examine genetic interaction between Ndel1 and Lis1, we mated Ndel1+/−/H9252 mice to Lis1−/−/H11001 mice or Lis1−/−/H11002 mice. Although Lis1+/− mice displayed their typical migration defect (17), Lis1−/−/H11001−/− mice showed overall fairly normal brain morphology. In contrast to single heterozygotes, the pyramidal cells in the CA3 region of the hippocampus were partially split in Ndel1+/−/H9252Lis1−/−/H11001−/− double heterozygotes (Fig. 4A). Lis1+/−/H11002−/− mice displayed severe migration defects in the hippocampus recognized by the splitting of pyramidal cells. These migration defects appeared to be slightly more severe in Ndel1+/−/H11001−/−Lis1−/−/H11002−/− double heterozygotes. These results suggested that Ndel1 and Lis1 genetically interacted during neuronal migration. The abnormal layering in the hippocampus was also clearly visualized by NeuN staining, a marker for terminal differentiation of neurons (Fig. 4B) (28). We assessed defects in cortical architecture and fragmentation of the subplate by immunohistochemical analysis with MAP2, which specifically identifies dendritic extensions of postmitotic neurons. In wild-type and Lis1−/−/H11002−/− embryos, MAP2-positive neuronal processes were arranged radially to form a tight, palisade-like pattern at the cortical plate (Fig. 4C, upper panels). MAP2 also stains the horizontal processes of subplate neurons (23) but not those in the intermediate zones (34). In contrast, radial and palisade staining of the subplate became irregular, and horizontal staining of the subplate was fragmented in Ndel1−/−/H9252−/−Lis1−/−/H11001−/−, and Ndel1+/−/H11002−/−Lis1−/−/H11002−/− double heterozygotes compared to Ndel1+/−/H11001−/−Lis1−/−/H11001−/−, and Lis1−/−/H11002−/− single heterozygotes. Chondroitin sulfate proteoglycan is distributed in the marginal zone and the subplate (37). This marker also revealed fragmentation and abnormal organization of the subplate from Ndel1−/−/H11002−/− (Fig. 4C, lower panels). Consistent with the biochemical interaction of LIS1 and NDEL1 (36), these developmental studies further suggest that Lis1 and Ndel1 genetically interact and synergistically regulate neuronal migration.

**BrdU birth dating analysis of Ndel1−/−null MEFs and genetic interaction with Lis1.** To confirm that the morphological defects observed in the several genetic combinations were due to abnormal neuronal migration, we performed quantitative BrdU birth dating analysis on each genetic combination. As the dose of Ndel1 was reduced, the distribution of labeled cells was shifted downward toward the ventricular zone in the cortex, and BrdU labeling was more diffusely localized (Fig. 5A, Ndel1−/−/H9252−/−Lis1−/−/H11001−/−) compared to Ndel1−/−/H9252−/−Lis1−/−/H11001−/− mutants. Although Lis1−/−/H9252−/−Lis1−/−/H11001−/− mutants displayed migration defects, additional heterozygous loss of NDEL1 significantly reduced the cell population that reached layer II (Fig. 5A, Ndel1−/−/H9252−/−Lis1−/−/H11001−/−). Although heterozygous loss of NDEL1 was not sufficient for the creation of a clear migration defect as in the Lis1−/−/H11001−/− mutants, combinatorial loss of Lis1−/−/H11001−/− and Lis1−/−/H11001−/− clearly led to a migration defect (Fig. 3B), suggesting that Lis1 and NDEL1 cooperatively function during neuronal migration in the cortex, as well as in the hippocampus.

**Apoptotic cell death in Ndel1−/−null MEFs and Lis1 mutants.** We observed mild reduction of the density of cells in the neocortex of the Ndel1+/−/H9252−/−Lis1−/−/H11001−/− mice and the Ndel1+/−/H11001−/−Lis1−/−/H11002−/− mice (Fig. 6A). To determine potential defects of corticogenesis due to reduction of LIS1 and/or NDEL1, we analyzed C-Neu immunoreactivity to label the large pyramidal neurons of layer 5 (43) and calbindin immunoreactivity to label the interneurons of layers 2 and 3 (24) in the adult cortex (8W) of these different crosses (Fig. 6B). C-Neu-positive cells that migrate at an early stage exhibited broader distribution in...
**DISCUSSION**

**Neuronal migration defects in Lis1 and Ndel1 mutant granule cells.** To define the mechanistic roles of NDEL1 and LIS1 in mammalian neuronal migration, we used mouse cerebellar granule neurons in an in vitro migration assay with wild-type or mutant neurons (17, 41). Migration distances in neurons transfected with RFP-CRE alone were positioned indistinguishably from untransfected neurons, suggesting that RFP-CRE transfection had no effect on migration (Fig. 8A and D). Overexpression of LIS1-GFP led to a mild increase in neuronal migration (Fig. 8A and D), whereas GFP-NDEL1 or GFP-NDE1 overexpression had no such effect (Fig. 8A, D). Lis1-null granule neurons displayed severe migration defects compared to wild-type neurons, characterized by a leftward shift of the bin distribution of migration distance, and the mean distance decreased by ~60% from the WT level (Fig. 8B and D). This migration defect due to Lis1 inactivation was efficiently rescued by exogenous expression of GFP-LIS1 (Fig. 8B and D) but not by GFP-NDEL1 or GFP-NDE1. Similar migration defects were observed in Ndel1-null neurons (Fig. 8C and D). This migration defect due to loss of Ndel1 was rescued by exogenous expression of GFP-NDEL1 (Fig. 8C and D). GFP-LIS1 expression only moderately rescued the migration defect. More interestingly, GFP-NDE1 expression partially but incompletely rescued the migration defect derived from loss of Ndel1. The reduced magnitude of rescue suggests that NDEL1 and NDE1 have similar functions, but NDEL1 might have a more crucial and distinct function. These data are consistent with the early embryonic lethality displayed by Ndel1-null mice compared to the viable phenotype of Ndel1-null mice, as well as the less-efficient recovery of disorganized microtubule network in Ndel1-null MEFs by GFP-NDE1 expression.

**Ndel1** is one of the mouse homologues of Nude from *Aspergillus*, which was identified as a multicopy suppressors of a mutation in the nudF gene, the *Aspergillus* homologue of LIS1 (6). NudE is also a homologue of the nuclear distribution protein RO11 (24) of *Neurospora crassa* and mitotic phosphoprotein 43 of *Xenopus laevis* (6). LIS1 and NDEL act in the CDHC pathway, which is the large molecular motor that translocates to the minus (−) ends of microtubules. Dynein, whose motor domain comprises six AAA modules and two potential mechanical levers, generates movement by a mechanism that is fundamentally different than that which underlies the motion of myosin and kinesin. CDHC is involved in numerous functions, including microtubule organization, vesicle transport (especially from the endoplasmic reticulum to the Golgi apparatus), chromosome separation, and nuclear distribution (12, 16, 45, 46).

To understand the in vivo role of NDEL1, we generated Ndel1-disrupted mice. Complete loss of NDEL1 caused embryonic lethality at the peri-implantation stage and a deficiency of cell proliferation in the inner cell mass. These data suggest that NDEL1-dependent regulation of CDHC is essential for proliferation and may play a role in important aspects of mitosis. Compared to Lis1+/− mice, which displayed clear neu-
neral migration defects (10, 17), Ndel1−/− mice did not exhibit obvious phenotypes. Further reduction of Ndel1 using a hypomorphic conditional allele resulted in mild neuronal migration defects. Double Lis1/Ndel1 mutants displayed more severe migration defect than single mutants, suggesting that Lis1 and Ndel1 genetically interact. Elevated apoptotic cell death during neurogenesis was observed in Lis1 mutants, whereas mutation of Ndel1 did not result in apoptosis. We also examined Lis1 and Ndel1 functions on dynein regulation and microtubule organization. Lis1- and Ndel1-null MEFs displayed similar disruption of the compact juxtanuclear Golgi complex of β-COP-positive vesicles. The aberrant distribution of β-COP-positive vesicles in Lis1-null MEFs was only rescued by exogenous introduction of GFP-LIS1, while the disrupted organization of β-COP-positive vesicles in Ndel1-null MEFs was efficiently rescued by GFP-NDE1, as well as GFP-NDE1 and 1, and it was partially rescued by GFP-LIS1. Lis1 disruption resulted in perinuclear accumulation of microtubules, whereas Ndel1 inactivation displayed amorphous distribution of microtubules. We also demonstrated that the disorganized microtubule network in Ndel1-null cells was completely rescued by GFP-NDE1 but only partially rescued by GFP-LIS1 and GFP-NDE1. These results were similar to those found for neuronal migration using cerebellar granule cell reaggregation assays. Neuronal migration defects displayed by Ndel1-null neurons were completely rescued by GFP-NDE1 but only partially rescued by GFP-LIS1 or GFP-NDE1 exogenous expression. Our data suggest that NDE1, LIS1, and NDE1 act in a common pathway to regulate dynein and altered neuronal cell fates. In contrast to NDE1, complete loss of Ndel1 resulted in perimplanation lethality, a phenotype similar to complete loss-of-function of Lis1 (17) and Cdhc (14). Ndel1 mutants more clearly displayed neuronal migration defect than Ndel1 mutants. These phenotypic differences between two homologues could be attributed to differences in the pattern and levels of expression during development (8, 29, 35, 36). NDE1 is expressed mainly in neuronal stem cells and is downregulated in the postmitotic neurons. In contrast, NDE1 is first expressed in the early embryo. Later, NDE1 is expressed in neuronal stem cells, and its expression continues in postmitotic neurons, suggesting that NDE1 plays a broader role during development than NDE1. Another nonexclusive possibility is that NDE1 has distinct molecular targets from NDE1. This possibility is supported by our observations that the disorganized microtubule network seen in the NDE1-null MEFs was not completely rescued by NDE1 expression. Partial rescue of neuronal migration defect in NDE1-null neurons by NDE1 introduction also supports this possibility. Further experiments with mutant mice for each of these components, which are now all available, as well as further biochemical studies, will provide more detailed explanations to address the similarities and differences in the roles.

FIG. 8. Migration distance in Lis1- or Ndel1-null neurons determined by reaggregation assay. Granular neurons were isolated from P3 neonatal pups and subjected to the neuronal migration reaggregation assay. Lis1 or Ndel1 was inactivated by CRE-mediated recombination. Cotransfected plasmids for rescue are shown on the left side. The migration distance of each neurons after 12 h was binned. (A) Wild-type neurons expressing RFP-CRE display normal migration distance compared to untransfected neurons. Cotransfection of GFP-LIS1 mildly enhanced migration, whereas GFP-NDE1 or GFP-NDE1 did not have obvious effect. (B and C) Lis1- or Ndel1-(C)-null neurons display a shift in the distribution of bins toward the right. Migration defects in Lis1-null neurons were only rescued by exogenous expression of GFP-LIS1. Migration defects in Ndel1-null neurons were rescued by exogenous expression of GFP-LIS1, GFP-NDE1, and GFP-NDE1, but GFP-LIS1 and GFP-NDE1 were less effective (see panel C). Mean migration distances are summarized at the bottom. n, Number of neurons measured for each examination. (D) Summary of mean migration distance of each genotype.
of LIS1, NDE1L1, and NDEL1 in the regulation of dynein function and microtubule organization, as well as their roles in proliferation, neurogenesis, and neuronal migration.

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