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Original Article

ORIGINAL ARTICLE

ADAM10-Initiated Release of Notch Intracellular Domain Regulates Microtubule Stability and Radial Migration of Cortical Neurons

Zhi Yang[1,2,†], Peng-Fei Li[1,3,†], Ren-Chao Chen[1,†], Jie Wang[1,3], Shaoran Wang[1,3], Ya Shen[1], Xiaohui Wu[4], Bing Fang[2], Xuewen Cheng[1,3] and Zhi-Qi Xiong[1,3]

[1]Institute of Neuroscience, State Key Laboratory of Neuroscience, CAS Center for Excellence in Brain Science and Intelligence Technology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China, [2]Shanghai Key Laboratory of Stomatology, Department of Oral and Cranio-Maxillofacial Science, Shanghai Ninth People's Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 200011, China, [3]University of Chinese Academy of Sciences, Beijing 100049, China and [4]Institute of Developmental Biology and Molecular Medicine, School of Life Science, Fudan University, Shanghai 200433, China

Address correspondence to Dr Zhi-Qi Xiong and Dr Xuewen Cheng, Institute of Neuroscience, State Key Laboratory of Neuroscience, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yueyang Road, Shanghai 200031, China. Email: xiongzhiqi@ion.ac.cn/cxwen999@ion.ac.cn

[†]These authors contributed equally to this work.

Abstract

Proper neuronal migration is orchestrated by combined membrane signal paradigms, whereas the role and mechanism of regulated intramembrane proteolysis (RIP) remain to be illustrated. We show here that the disintegrin and metalloprotease-domain containing protein 10 (ADAM10) regulates cortical neurons migration by initiating the RIP of Notch. We found that Notch intracellular domain (NICD) significantly rescued the migration defect of ADAM10-deficient neurons. Moreover, ADAM10 deficiency led to reduced neuronal motility and disrupted microtubule (MT) structure, which were associated with downregulated expression of acetylated tubulin and MT-associated proteins. Specifically, the NICD/RBPJ complex bound directly to the promoter, and regulated the neuronal expression level of doublecortin (DCX), a modulator of the MT cytoskeleton. Functionally, DCX overexpression largely restored neuron motility and reversed migration defect caused by ADAM10 knockout. Taken together, these findings demonstrate the direct requirement of ADAM10 in cortical radial migration and reveal the underlying mechanism by linking ADAM10-initiated RIP of Notch to the regulation of MT cytoskeleton through transcriptional control of *Dcx* expression.

Key words: ADAM10, DCX, microtubule, Notch, radial migration

Introduction

Radial migration of cortical neurons from their neurogenic zone to defined layers is essential for normal morphogenesis and circuit formation (Bielas et al. 2004; Rakic 2006; Ayala et al. 2007; Cooper 2014). Impaired migration is associated with many inherited neurological disorders such as mental retardation and epilepsy (Bielas et al. 2004; Valiente and Marin 2010). In mouse cerebral cortex, migration of late-born neurons takes several days to reach the superficial layer, making it important to equip newborn neurons with multiple strategies to sense surrounding circumstance and precisely orchestrate intracellular molecular machinery for migration. Membrane-associated signal events play crucial roles in the control of neuronal radial migration. Some transduction paradigms, such as binding of ligands to membrane receptors and interactions between cell adhesion molecules, have been illustrated as critical mechanisms underlying migration (Ayala et al. 2007; Cooper 2014), but some others remain obscure. Regulated intramembrane proteolysis (RIP), in which a transmembrane protein releases its cytoplasmic domain via sequential proteolytic cleavages (Murphy 2008), was recently implicated in radial migration. The Notch intracellular domain (NICD), an RIP product of the Notch receptor, appears to be essential for proper radial migration of cortical neurons (Hashimoto-Torii et al. 2008), suggesting that RIP regulates radial migration. Moreover, eliminating presenilin 1 (PSEN1), a γ-secretase subunit required for intracellular cleavage of either β-amyloid precursor protein (APP) or Notch, caused similar migration defects (Louvi et al. 2004). However, how RIP functions in regulating radial migration remain largely unknown.

The transmembrane disintegrin and metalloprotease-domain containing protein 10 (ADAM10, also known as kuzbanian), highly expressed in developmental central nervous system (Jorissen et al. 2010), is a critical protease that initiates RIP by juxtamembrane cleavage of membrane substrates to shed the ectodomain thereby enabling intracellular cleavage by γ-secretase (Murphy 2008). Removal of ADAM10 in neural progenitor cells by Nestin-Cre impairs neuron migration (Jorissen et al. 2010). However, whether ADAM10-initiated RIP directly functions in migrating neuron requires stage-specific dissection. In neural progenitor cells, ADAM10 is the major protease for extracellular cleavage of transmembrane targets such as Notch, APP, and *N*-Cadherin (Hartmann et al. 2002; Reiss et al. 2005; Kuhn et al. 2010), while does ADAM10-initiated RIP of these proteins remain conserved in migrating neurons needs further examination, since the RIP of them by ADAM8, ADAM9, and ADAM17 (TACE) has also been reported (Murphy 2008). Moreover, whether the assumed regulation of neuron migration by ADAM10 mainly depends on the RIP of the above substrates, or otherwise through the RIP-independent interaction with other membrane partners like Ephrin and integrin (Schmid and Anton 2003; Senturk et al. 2011)? Nevertheless, ADAM10-associated membrane signaling enables newborn neurons with a differential strategy to sense environmental stimuli and direct intracellular migration machinery. Therefore, elucidating the function and mechanism of ADAM10 in neuron migration would be an interesting question.

The microtubule (MT) cytoskeleton is critical for neuronal migration (Feng and Walsh 2001; Heng et al. 2010). Regulation of MT dynamics by MT-associated proteins (MAPs) controls neuron motility and cortical lamination. Intensive studies have shown that different posttranslational modifications of MAPs, such as phosphorylation and glycosylation, exert diverse effects on MT regulation and radial migration (Brugg and Matus 1991; Hiesberger et al. 1999; Tanaka et al. 2004b). Recently, doublecortin (DCX) was found to be regulated by SnoN1-FOXO1 repressor complex at transcriptional level during the migration of granule cells in cerebellum (Huynh et al. 2011). Whether transcriptional regulation of MAPs is also involved, and the potential relationship between MT dynamics and ADAM10-initiated RIP in the regulation of neuron migration in cerebral cortex remains to be established.

In the present study, we identified ADAM10 as a critical component in the regulation of radial migration. Selective inactivation of ADAM10 in newborn cortical neurons disrupted the normal migration process. Moreover, we found that ADAM10 exerts its effect on radial migration predominantly by initiating the RIP of Notch to release NICD, which in turn promoted the expression of MAP genes like *Dcx*, thus modulating MT structure and neuronal motility. Our work reveals a new function of ADAM10-initiated RIP in the regulation of neuronal development and provides new insight into the molecular mechanisms of Notch signaling and RIP-mediated regulation of neuronal migration.

Materials and Methods

Mice and Constructs

The *Adam10*$^{f/f}$ mice were generated as described previously (Tian et al. 2008). For nonelectroporation knockout, the floxed mice were crossed with *Dcx*-CreER^{T2} mice (Cheng et al. 2011). Mice were raised at 22 °C in isolated-air-supply cages with clean water and food in a barrier environment with an auto day/night switch. Rearing, surgery, and euthanasia procedures were approved by the Institutional Animal Care and Use Committee (IACUC).

The expression constructs used were cloned into plasmids with 2 kinds of promoters, CAG promoter for Cre, GFP, or loxp-STOP-loxP-ORF (termed LSL-ORF), and mouse *Dcx* promoter for pDcx-Cre engineered from pDcx-Cre-iGFP (Franco et al. 2011). Template plasmids of AD10(E385A), AD10(ΔMP) (Addgene number: #19138 and #19137), and APP_C60 were obtained from Addgene (Grillet et al. 2016), and pCAG-Cre-iGFP was a gift from Dr Xiao-Bing Yuan, pDcx-Cre-iGFP from Dr Ulrich Mueller (Franco et al. 2011), Cdh2 constructs and pNeuroD1-Cre from Prof. J. Cooper (Grillet et al. 2016). To knockdown *Adam10*, *Notch1*, or *Rbpj*, their shRNA oligos were inserted into a p*Super-basic* vector (termed sh.gene) or loxp-STOP-loxp engineered construct (termed LSL-sh.gene) with H1 promoter. The targeting sequences of used shRNA are *ADAM10* shRNA1, 5′- GCTCATGAAGTTGGACATA-3′; *ADAM10* shRNA2 (sh.AD10), 5′-GCCAAGTGCTTGAGAAGAA-3′; *Notch1* shRNA (sh.Nch1), 5′-GCCCTTTGAGTCTTCATACAT-3′; and Rbpj shRNA (sh.Rbpj), 5′-GCATGGCACTCCCAAGATTGA-3′. For more details, see Supplementary Experimental Procedures.

In Utero Electroporation

Pregnant mice were used for in utero electroporation at indicated times with ECM-830 square-pulse generator (BTX). In loss-of-function experiments, shRNA, p*CAG*-Cre, or p*Dcx*-Cre was mixed with pCAG-LSL-GFP reporter at a ratio of 10:1 (3:0.3 μg/μL). In rescue experiments, p*Dcx*-Cre, p*CAG*-LSL-GFP, and indicated LSL-ORF constructs were mixed at a ratio of 3:0.3:2 (3:0.3:2 μg/μL).

The electroporated brains were harvested at indicated times and fixed in 4% paraformaldehyde (PFA) solution for 24 h, then dehydrated in a 20% sucrose PBS solution for another 24 h. Brain slices were cryosectioned at a thickness of 50 μm for immunostaining and imaging. At least 4 embryos from separate electroporations were used at each time point or group.

BrdU Labeling and Tamoxifen Induction

To activate CreER^{T2}, tamoxifen (20 mg/mL, diluted in corn oil, Sigma) was administered for 4 consecutive days at a dose of 2 mg/40 g (body weight) using a gavage needle following the indicated time paradigm. BrdU was injected intraperitoneally at a dose of 50 mg/kg body weight. To trace neuronal migration, BrdU was administered intraperitoneally twice within 1 day at E15.5. To analyze the proliferation of neural progenitors, mice receiving in utero electroporation at E14.5 were injected 48 h later with BrdU, and sacrificed 2 h later for the preparation of brain slices.

Immunofluorescence and Imaging

Immunostaining was performed using 50 μm coronal slices or coverslips as described previously. Coverslip-cultured neurons were pretreated with 4% PFA for 15 min on ice. The brain slices or coverslips were penetrated with 0.25% Triton X-100 for 45 min at room temperature (RT). After washing with 1× PBS, slices were blocked in PBS with 5% BSA and 0.1% Triton X-100 for 45 min, and then incubated with primary antibodies overnight at 4 °C. The antibodies used were mouse anti-Ac-α-tubulin, mouse anti-Tyr-Tubulin and mouse anti-α-Tubulin (Sigma); rabbit anti-Tbr1, rat anti-Ctip2, rat anti-BrdU (Abcam); rabbit anti-Cux1, and goat anti-DCX antibodies (Santa Cruz); and rabbit anti-GFP antibody (Invitrogen). The next day, slices were subsequently washed 3 times in PBS for 15 min, and then incubated with appropriate secondary antibodies for 2 h at RT, followed by Hoechst staining and 4 washes in PBS, and finally mounted on glass slides. Before immunostaining with anti-BrdU antibody (Abcam), the brain slices were treated with 2 N HCl for 30 min at 37 °C, followed by 10 min neutralization in 0.1 M borate buffer (pH 8.5). Z-stacks of images taken at 3 μm intervals were acquired and photographed under the ×10 objective lens of a Nikon A1R confocal microscope equipped with a 335-nm, 488-nm, and 543-nm laser. To quantify neuron distribution using Image-J software, brain slices from the somatosensory medial cortical region were divided into 10 equal-wide bins as marked in the images of migration assay, and the number of GFP$^+$ neurons within each bin was calculated and presented as % of the total number of cells in all of the 10 bins.

For cultured cells, Z-stacks of images were taken with ×20 objective lens at 1 μm intervals for imaging of cells, or taken with ×60 objective lens at 0.4 μm intervals for imaging of Tyr-Tubulin. For the imaging of MT structure of neuron soma at higher resolution, cells were fixed with 4% PFA plus 0.2% glutaraldehyde for 15 min before immunostaining with anti-Ac-Tubulin antibody. A ×100 TIRF objective lens were used to take microscope images (X–Y resolution: 0.049718 μm/pixel). Fluorescent beads with a diameter of 0.1 μm were imaged using the same settings to generate PSF (point spread function), which was used for deconvolution of all the neuron images by Huygens software. The output images were than z-projected using Image-J. The MT structure of each cell was traced using Neurolucida software, and the total MT fiber length and number in soma region were quantified.

Cell Culture, Transfection, and Transwell Assay

Mouse primary cortical neurons were cultured using E18 cortices as described previously (Wu et al. 2012). For transfection, 5×10^6 neurons were mixed with 10 μg plasmid DNA and electroporated using the Amaxa Nucleofector system (Lonza). Neurons were plated in PDL-pretreated plates (Corning), cultured in Neurobasal medium supplemented with 2% B27 (Gibco) and incubated in a CO_2 incubator at 37 °C.

For transwell assay, E14.5 ADAM10$^{f/f}$ mice electroporated with p*Dcx*-Cre and GFP reporter constructs were dissected and GFP-labeled cortical neurons were isolated 3 days after electroporation. The GFP$^+$ neurons were sorted and plated at a density of 2.5×10^5 cell/well into chambers of the Boyden transwell system (5 μm, Corning) coated with PDL (0.01 mg/mL) and Laminin (1 μg/mL). Forty-eight hours later, neurons were fixed with 4% PFA, and cells remaining on the surface of the inserted membrane were carefully removed. Following immunostaining with GFP antibody, the GFP$^+$ cells that migrated across the pores of the transwell were imaged and quantified.

Western Blot

Mouse brain tissue was homogenized using a Tissuelyzer (Jingxin) or Polytron PT 1300 (Kinematica) in lysis buffer (Tris-Cl 50 mM, pH 7.4, NaCl 150 mM, EDTA 50 mM, SDS 0.5%, Triton X-100 1%, plus protease inhibitor cocktail). For cultured neurons, lysate was prepared directly using sample loading buffer containing 1% SDS. Protein concentration was measured using the BCA reagent (Tiangen) and read with a Spectromax microplate reader (Molecular Devices). In all, 30–40 μg protein was loaded into each lane and run in an SDS-PAGE gel and then transferred onto PVDF membrane (Millipore), and followed by blocking with TBS with 0.05% Triton X-100 plus 5% milk and then incubation with appropriate primary and matched secondary antibodies. The antibodies used were rabbit anti-ADAM10, mouse anti-α-tubulin, mouse anti-ac-α-Tubulin, and Mouse anti-β-tubulin (Sigma); rabbit anti-Notch1, rabbit anti-NICD, rabbit anti-RBPJ (Cell Signal); mouse anti-β-actin, goat anti-DCX (Santa Cruz); mouse anti-LC3b, mouse anti-MAP1B, mouse anti-MAP2, and mouse anti-Tau1 (Abclonal). Finally, protein signal was detected using a chemoluminescent kit (Pierce/Thermo).

RNA Extraction and Real-Time PCR

Total RNA was extracted from mouse cortical tissue or cultured cells using Trizol reagent (Invitrogen) and reverse transcribed in a 96-well temperature cycler (ABI) with oligo-dT$_{(18)}$ using an M-MLV Reverse Transcriptase kit (Invitrogen). For real-time PCR, a SYBR Premix Ex Taq kit (Takara) was used to prepare PCR solution, and reactions were run on LightCycler 480 (Roche) with standard program. The real-time PCR primer sequences used were *adam10* forward, AAGCAGTGCAGTCCGAGTCAA, reverse, CCATTGCATA TCCCTTCCTTTG; *dcx* forward, TCAAGCCAGAGAGAACAAGGACTT, reverse, GCTCGAAAGAGTGGGCTGTTT; *gapdh* forward, CTGCCCA GAACATCATCCCT, reverse, TGAAGTCGCAGGAGACAACC; Map2 forward, GCAAGGATAGTTCAAGTAGTCA, reverse, CGAAGG TGGCAGATTAGC; Tau1 forward, AGATTGGCTCCTTGGATAA, reverse, GGAAGACACATTGCTGAG.

Chromatin Immunoprecipitation Analysis and Luciferase Assay

Chromatin immunoprecipitation (ChIP) analysis was carried out using the EZ-ChIP kit according to the manufacturer's protocol (Millipore). DIV3 cortical neurons were crosslinked with formaldehyde (1% final concentration) for 15 min at RT. After quenching with glycine and washing with PBS, cells were

collected and redissolved in 1 mL lysis buffer, then sonicated (Bioruptor, Grade L, 20 s on/25 s off, 15 times) to generate DNA fragments with an average length of 200–500 bp. The chromatin was diluted and incubated overnight at 4 °C with the ChIP antibodies (1 μg/mL of rabbit anti-NICD, rabbit anti-RBPJ, and rabbit IgG [Cell Signal] and rabbit anti-GFP [Invitrogen]). The next day, protein A beads (Roche) were added and incubated for an additional 2 h. The pellets were subsequently washed with several solutions and eluted in TE buffer (10 mM Tris-Cl, pH 8.0, 1 mM EDTA). Supernatants were decrosslinked at 65 °C for 5 h and digested with proteinase K for another 2 h. The DNA was then extracted using a DNA purification column and resolved in TE buffer for real-time PCR using the LightCycler 480 System (Roche). The primers used were RBS2.2 forward, GGCTTTGGTT GTATTATTGGGTT, reverse, ATTGCTTAGGTGTATTGATGGAGG; RBS 3.8 forward, AGGAGTGGGAAGGTGAAACATT, reverse, CCT CTGTTCTGCTTCTTGGCTA.

Luciferase assays were performed as described previously (Hu et al. 2011). Briefly, *Dcx/DCX* promoter or their mutant cloned from mouse/human genome BAC DNA was inserted into the pGL3-basic vector to drive firefly luciferase transcription. Cortical neurons were electroporated to deliver plasmids of luciferase, internal control (p*RL*-SV40), and regulator genes (shRNA, Cre, or NICD) at a ratio of 5:1:5, and cultured in Neuralbasal medium plus 2% B27 in 24-well plates. Thirty hours later, cells were collected, lysed with passive lysis buffer, and centrifuged. Luciferase luminescence of supernatant was examined using a Dual-Luciferase Reporter Assay System (Promega) in a Flexstation microplate reader (Molecular Devices).

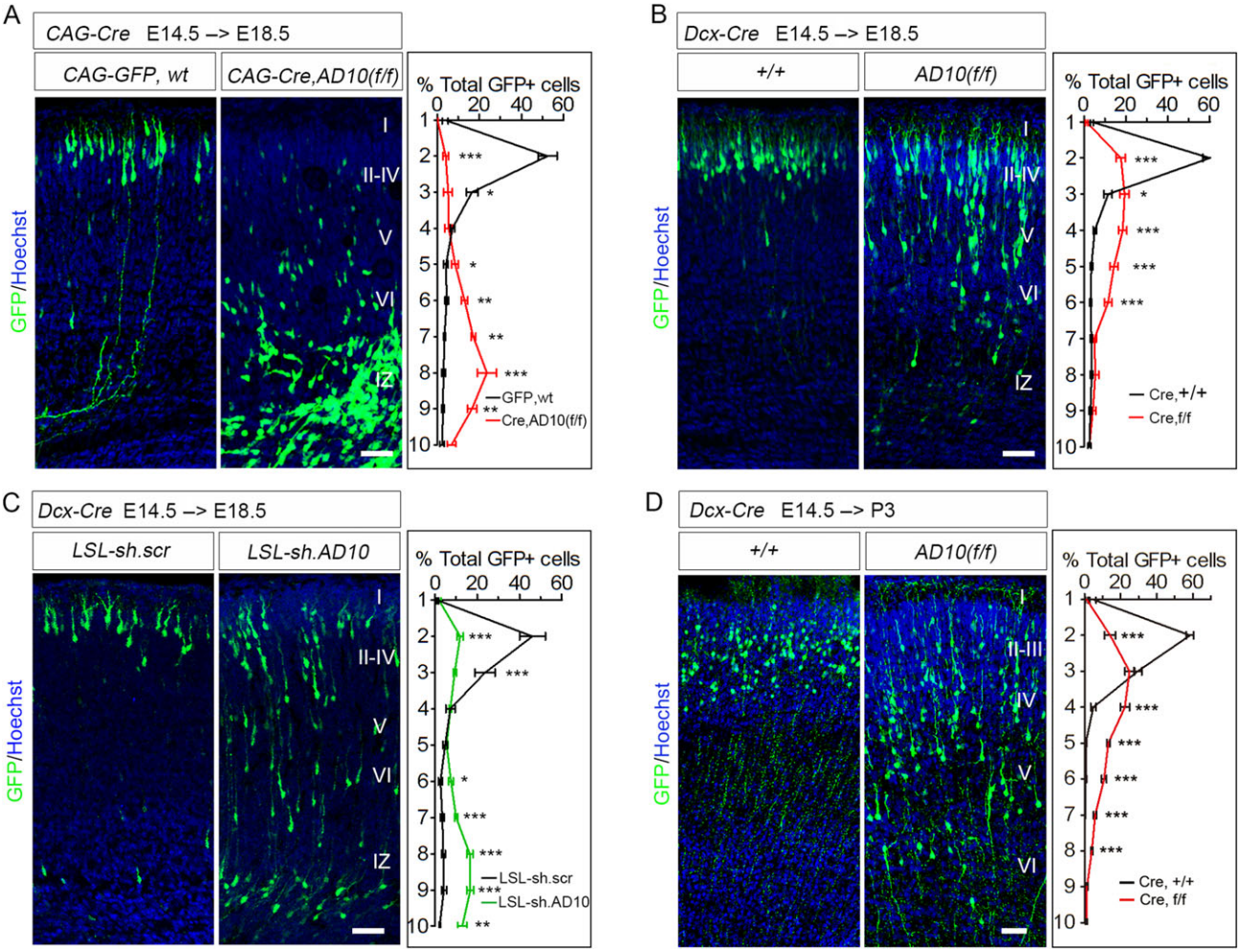

Figure 1. Direct requirement of ADAM10 for cortical radial migration. (*A*) Knockout of *Adam10* by pCAG-Cre impairs radial migration. The $Adam10^{f/f}$ embryos or WT littermates were electroporated with pCAG-Cre and LSL-GFP at E14.5, coronal sections of brains at E18.5 were analyzed. Quantitative analysis of cell distribution is shown in the right panel. (*B*) Conditional knockout of *Adam10* by p*Dcx*-Cre in newborn neurons leads to their migration defects. The $Adam10^{f/f}$ embryos or control littermates (+/+) were electroporated with p*Dcx*-Cre and LSL-GFP at E14.5, coronal sections of brains at E18.5 were analyzed. Quantitative analysis of cell distribution is shown in the right panel ($n = 5$, control; $n = 6$, *AD10*.flox). (*C*) Conditional knockdown of *Adam10* by p*Dcx*-Cre in newborn neurons leads to their migration defects. A conditional *Adam10* shRNA construct (*LSL-sh.AD10*) or scramble shRNA plasmid (*LSL-sh.scr*) was co-electroporated with p*Dcx*-Cre and LSL-GFP into mouse ventricles at E14.5. Coronal sections of brains at E18.5 were analyzed. Quantitative analysis of cell distribution is shown in the right panel ($n = 3$, control; $n = 5$, *sh.AD10* group). (*D*) Conditional knockout of *Adam10* by p*Dcx*-Cre in newborn neurons leads to their migration defects. The $Adam10^{f/f}$ embryos or control littermates (+/+) were electroporated with p*Dcx*-Cre and LSL-GFP at E14.5, coronal sections of brains at P3 were analyzed. Quantitative analysis of cell distribution is shown in the right panel ($n = 4$ for each group). (*E*, *F*) ADAM10 deficiency in migrating neurons does not change their subtype neuronal identity. Costaining of GFP and Cux1 (*E*), Ctip2 (*F*, left), or Tbr1 (*F*, right) using p*Dcx*-Cre electroporated $Adam10^{+/+}$ or $Adam10^{f/f}$ brain slices at P3. e1–e4 and f1–f2 are high magnification pictures of selected region indicated in the above panel. Scale bar, 50 μm.

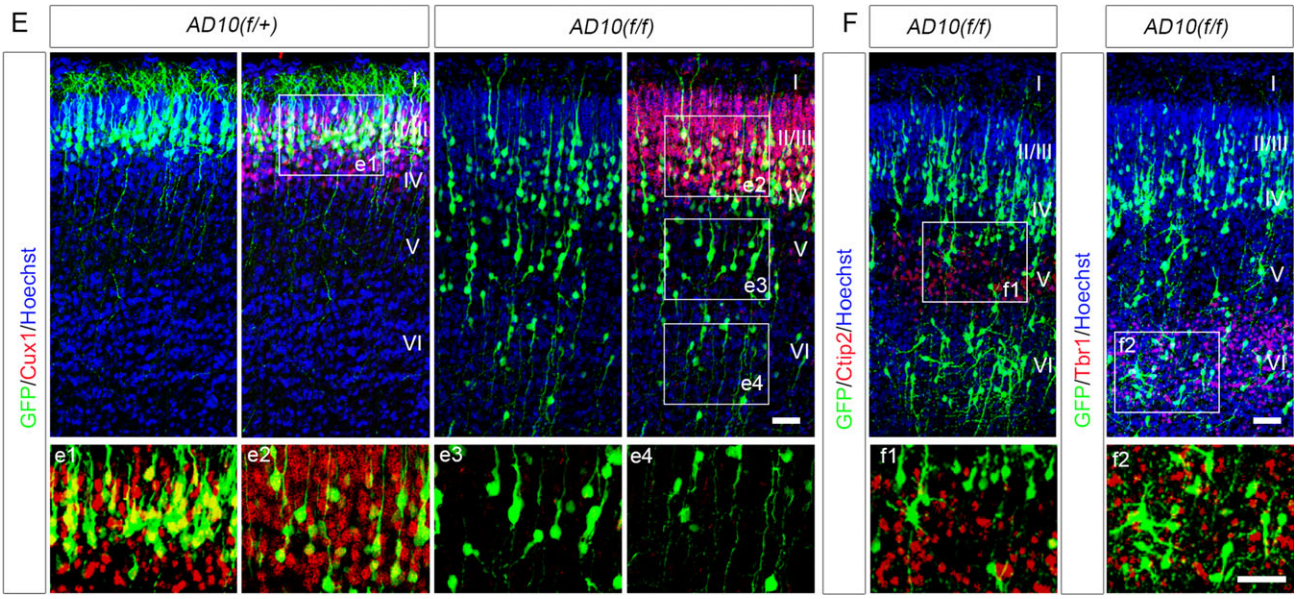

Figure 1. *Continued.*

Statistical Analysis

All statistical analyses were done using Student's *t*-test (2 groups) or ANOVA (multiple groups). All data are presented as mean ± standard error of the mean (SEM). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

Results

ADAM10 Is Directly Required for Radial Migration of Cortical Neurons

To determine at which stages in neuronal development that ADAM10 may function, we first examined its expression in developing mouse brain. Both the precursor and mature forms of ADAM10 protein were abundantly expressed in cerebral cortex from embryonic day (E) 15 to postnatal day (P) 3 (Supplementary Fig. 1*a*). Moreover, immunostaining revealed that ADAM10 was expressed not only in the ventricular zone (VZ), but also in the subventricular zone (SVZ) and cortical plate (CP) from E16.5 to P0 (Supplementary Fig. 1*c*), indicating that ADAM10 may play a role in radial migration.

To address the role of ADAM10 in cortical radial migration, we electroporated *Adam10* shRNA construct together with a GFP plasmid into mouse embryos at E14.5 (Supplementary Fig. 1*b*). At E18.5, neurons transfected with *Adam10* shRNA were mostly detained in the SVZ/intermediate zone (IZ) region, whereas the neurons transfected with "scramble" shRNA migrated into the superficial layer of the CP (Supplementary Fig. 1*d*), suggesting that ADAM10 knockdown impaired radial migration. To rule out the possible off-target effect of shRNA-mediated knockdown, we electroporated a Cre construct driven by the CAG promoter (referred as *pCAG*-Cre) into E14.5 *Adam10*$^{f/f}$ embryos (Tian et al. 2008). Four days later, ADAM10-knockout (cKO) neurons in *Adam10*$^{f/f}$ mice were severely retarded, but neurons in control littermates migrated normally (Fig. 1A), demonstrating again that ADAM10 is critical for neuron migration.

As neither shRNA nor pCAG-Cre mediated removal of ADAM10 could dissect the newborn neuron stage from neural progenitors, the observed migration defect may be a secondary effect due to precocious differentiation of ADAM10-deficient progenitors (Jorissen et al. 2010). Therefore, we used Dcx promoter driving Cre (pDcx-Cre) to knockout ADAM10 selectively in newborn cortical neurons (Francis et al. 1999; Franco et al. 2011), of which the specificity was verified by the expression of GFP reporter (pCAG-LSL-GFP) 48 h after electroporation (Supplementary Fig. 2). We then delivered this construct into *Adam10*$^{f/f}$ and control embryos at E14.5. Four days later, a large portion of GFP^{+} cells were found to be scattered from layer VI to layer IV in the cortex of *Adam10*$^{f/f}$ brain, while most GFP^{+} neurons had migrated into layer II/III in control cortex (Fig. 1B), suggesting that ADAM10 is directly required for radial migration. Moreover, we did conditional knockdown of ADAM10 in newborn cortical neurons by using pDcx-Cre to control the expression of a loxP-STOP-loxP flanked shRNA construct (pH1-*LSL-sh.AD10*). We also found a significant migration defect, with more ADAM10-deficient neurons sequestered in IZ (Fig. 1C), possibly due to more rapid effect on the deficiency of ADAM10 protein by shRNA-mediated knockdown of *Adam10* mRNA. Furthermore, the migration defect of Adam10-cKO neurons was still observed at P3, and the abnormal neuron positioning was kept with a similar pattern to that at E18.5 (Fig. 1D), suggesting that ADAM10-cKO caused neuron migration defect is not a temporal delay, but a long-term positioning change.

To rule out the possibility that the neuronal migration defects were due to a change in neuronal identity, we performed immunostaining with antibodies against layer-specific markers Cux1 (layers II–IV), Ctip2 (layer V), and Tbr1 (layer VI). The majority of the misplaced neurons still expressed Cux1 (Fig. 1E). Some of the Cux1^{+} neurons did not show GFP expression, which may be those pre-existing Cux1^{+} cells resided at bottom layer in the secondary somatosensory cortex (S2) or insular cortex (IC), which can also be found in other report (Franco et al. 2011). Moreover, none of the GFP-labeled neurons was found to colocalize with Ctip2 or Tbr1 (Fig. 1F), suggesting that ADAM10 knockout may not change the identity of newborn cortical neuron. Taken together, these results demonstrated that ADAM10 is directly required for the radial migration of newborn cortical neurons.

Tamoxifen-Induced Knockout of ADAM10 in Cortical Neurons Leads to Their Migration Defect

Electroporation-mediated knockout only affects a small proportion of neurons in the cortex. To determine whether deleting

ADAM10 in a greater number of neurons similarly affected radial migration, we crossed *Adam10*$^{f/f}$ mice to *Dcx*-CreER^{T2} mice (Cheng et al. 2011). Tamoxifen was administered (2 mg/40 g body weight) using a gavage needle once a day from E14.5 to E17.5. Two days later, immunostaining showed that ADAM10 was knocked out in majority of the neurons in CP, whereas ADAM10 expression in SVZ region was not obviously affected (Fig. 2A).

To analyze the function of ADAM10 in neuronal migration in *Dcx*-CreER^{T2}:*Adam10*$^{f/f}$ mice, we pulse-labeled newborn neurons with BrdU (50 mg/kg body weight) at E15.5 during tamoxifen induction. At E19.5, we found BrdU^{+} neurons in control mice (*Dcx*-CreERT2(–)) migrated normally to an area within cortical layers II–IV, suggesting that tamoxifen administration does not affect neuron migration. In contrast, we found that the majority of BrdU^{+} neurons in *Adam10*-cKO brains were dispersed in layers V and VI (Fig. 2B), consistent with the phenotype observed in the above embryo electroporation experiments (Fig. 1B–D). As tamoxifen-induced CreERT2 activation in DCX-expressing neurons takes places in the entire cerebral cortex, this result reconfirms the critical function of ADAM10 in cortical radial migration.

ADAM10-Mediated Proteolytic Cleavage of Notch is Critical for the Regulation of Radial Migration

ADAM10 is a transmembrane protein containing an extracellular metalloprotease domain and an disintegrin domain (Murphy 2008). It is critical to determine whether the function of ADAM10 in radial migration was reliant on the RIP of transmembrane substrates or elsewise its interaction with membrane partners. To address this, we generated constructs of wild-type (WT) AD10, protease domain-truncated AD10(ΔMP) and catalytic site-mutated AD10(E385A) with their expression conditioned by Loxp-STOP-Loxp sequence (Grillet et al. 2016). Each construct was then delivered into ADAM10$^{f/f}$ embryonic cerebral cortex together with p*Dcx*-Cre and pCAG-LSL-GFP by electroporation. Replenished wt ADAM10 led to a complete reversal of the migration defects, whereas neither AD10(ΔMP) nor AD10(E385A) exhibited any mitigating effects (Fig. 3A,B). Therefore, the proteolytic activity of ADAM10 is essential for its function in the regulation of radial migration.

The requirement of protease activity implies that ADAM10 controls neuronal migration through proteolysis of its substrate(s). Previous studies have identified ADAM10 as a major protease initiating the RIP of a variety of membrane proteins, including Ephrins, APP, L1 adhesion molecule, *N*-cadherin, and Notch (Murphy 2008). Furthermore, Notch, APP, and *N*-cadherin have each been implicated in the regulation of neuronal migration in recent years (Young-Pearse et al. 2007; Hashimoto-Torii et al. 2008; Franco et al. 2011). To determine whether one of these proteins mediates the function of ADAM10 in radial migration, we generated LSL-conditional expressing constructs inserted with different ORFs encoding intracellular domain of these molecules, including NICD, *N*-cadherin intracellular domain Cdh2_CTF1, and intracellular domain APP_C60. Co-electroporation of each of these constructs together with p*Dcx*-Cre and pCAG-LSL-GFP into *Adam10*$^{f/f}$ embryos demonstrated that only replenished NICD into ADAM10-deleted neurons significantly rescued the migration defect (Fig. 3C,D), suggesting that Notch is a critical mediator of ADAM10 in regulating radial migration.

To confirm this was indeed the case, we examined whether the cleavage of Notch was impaired through p*Dcx*-Cre-mediated knockout of ADAM10 (Franco et al. 2011). The GFP-expressing cortical tissue blocks of *Adam10f/f* brain were harvested 48 h after electroporation with p*Dcx*-Cre and pCAG-LSL-GFP constructs, and the GFP^{+} neurons were collected by FACS sorting. Conditional removal of ADAM10 in cortical neurons led to a significant decrease in NICD protein (Fig. 3E), indicating that ADAM10 is the major protease that initiating the RIP of Notch. This result supports the notion that the function of ADAM10 in radial migration depends on its RIP product NICD.

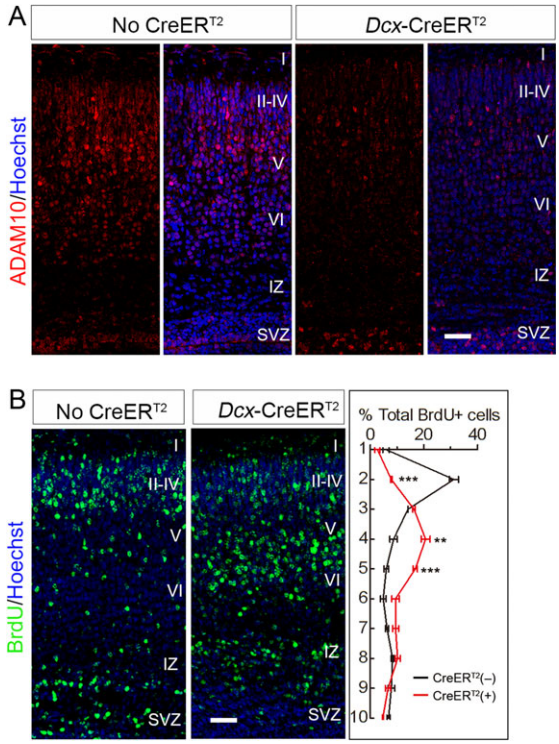

Figure 2. Tamoxifen-induced knockout of ADAM10 in cortical neurons leads to their migration defect. (A) Tamoxifen induction leads to significant removal of ADAM10 in CP of *Dcx*-CreER^{T2}/*Adam10*$^{f/f}$ mice. The pregnant *Dcx*-CreER^{T2}/*Adam10*$^{f/f}$ or control mice received tamoxifen (gavage injection) once a day from E14.5 to E17.5 and were sacrificed 2 days later. Coronal sections of E19.5 brains were immunostained with ADAM10 antibody (red) and cell nuclei were labeled by Hoechst (blue). (B) Tamoxifen-induced knockout of Adam10 caused migration defects shown by BrdU labeling in *Dcx*-CreER^{T2}/*Adam10*$^{f/f}$ mice at E19.5. Quantitative analysis of neural positions across the cortex was shown at the right (n = 3 for each group). Scale bar, 50 μm.

ADAM10 Deficiency Reduces Neuronal Motility and Alters MT Cytoskeleton Dynamics

Newborn neurons generated at the VZ/SVZ undergo a mutipolar–bipolar transition prior to glia-dependent locomotion (Valiente and Marin 2010; Cooper 2014). This involves re-organization of the MT cytoskeleton to correctly polarize migration and ensure transport of lipids, organelles, and proteins to the leading edge (Feng and Walsh 2001; Yokota et al. 2009; Eom et al. 2014). Using an in vitro transwell assay, we found that the motility of newborn cortical neurons was significantly impaired in the absence of ADAM10 (Fig. 4A,B). Moreover, deletion of ADAM10 produced more branches of MT structure in neurite terminals of cultured early cortical neurons (Fig. 4C,D), a phenomenon associated with reduced stability of MT structure (Wu et al. 2012). These results

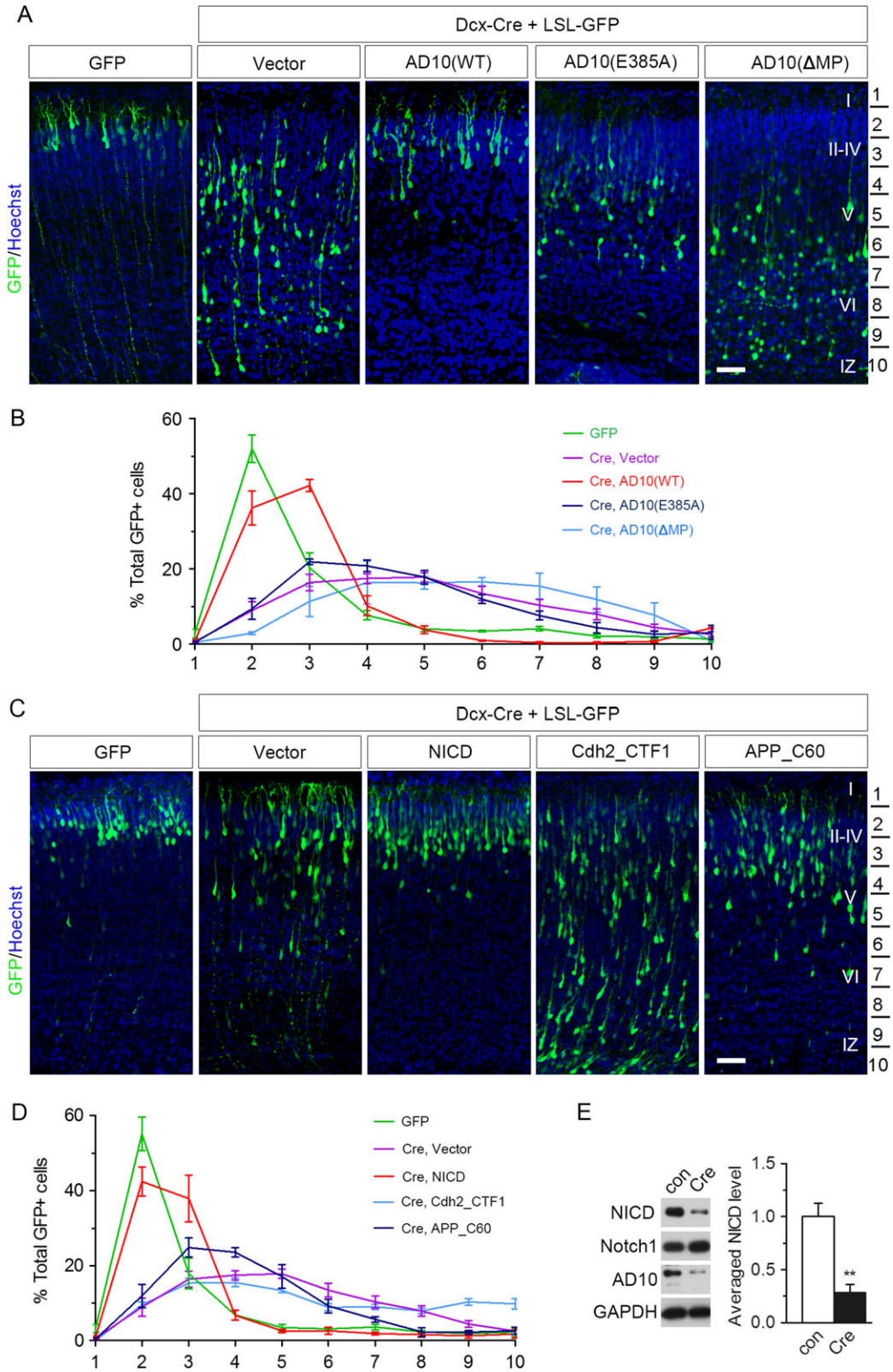

Figure 3. ADAM10-mediated proteolytic cleavage of Notch is critical for the regulation of radial migration. (*A*, *B*) Protease activity is essential for ADAM10 in regulating radial migration. Control and *Adam10*$^{f/f}$ embryos were electroporated with pDcx-Cre, LSL-GFP, and pCAG-LSL inserted with indicated Adam10 wt or mutants at E14.5 and sacrificed 4 days later. Quantitative analysis of cell distribution is shown in (*B*) ($n = 4$ for each group). (*C*, *D*) ADAM10-initiated RIP of Notch is critical for the regulation of neuronal migration. Control and *Adam10*$^{f/f}$ embryos were electroporated with p*Dcx*-Cre, LSL-GFP, and pCAG-LSL constructs inserted with indicated ADAM10 substrates at E14.5 and sacrificed 4 days later. Quantitative analysis of cell distribution is shown in (*D*) ($n = 3$ for the first 2 groups; $n = 4$ for the rest). (*E*) Conditional knocking out ADAM10 in cortical neurons reduced the level of NICD in mice brain. Cortical tissue blocks from E16.5 control and p*Dcx-Cre* plasmid electroporated *Adam10*$^{f/f}$ mice were isolated and the protein levels of ADAM10 and NICD were analyzed by western blot. Scale bar, 50 μm.

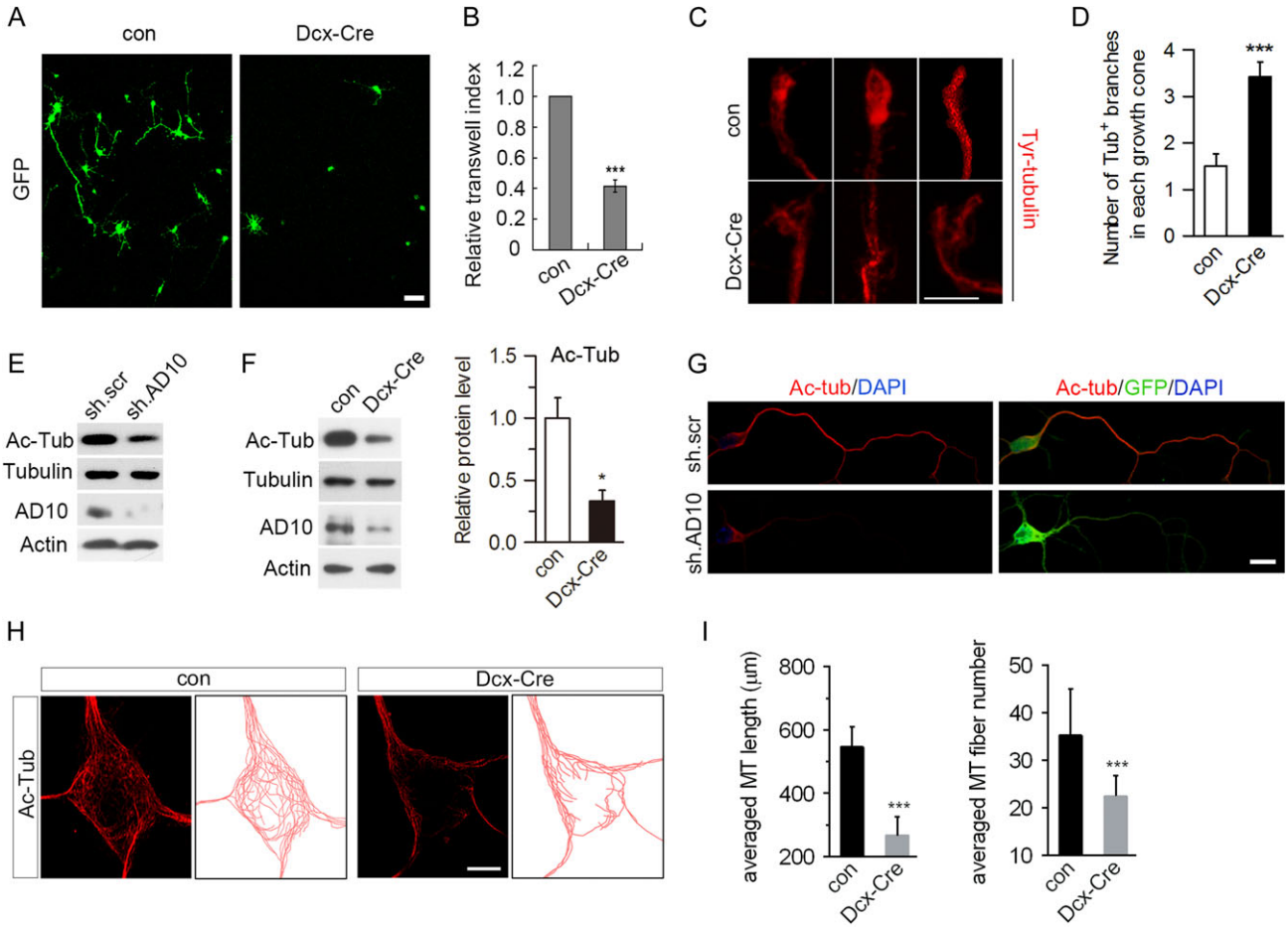

Figure 4. ADAM10 deficiency alters neuron motility and MT structure. (*A*, *B*) Transwell assay of GFP$^+$ neurons derived from *Adam10$^{f/f}$* cortices electroporated with control and *Dcx*-Cre constructs (left). The number of migrating GFP$^+$ cells found in the target well was counted and analyzed (*B*) ($n = 4$, ***$P < 0.001$ by Student's *t*-test). (*C*, *D*) Immunostaining of tyrsinated tubulin (Tyr-Tub) showed more branches or comets of MT structure at neurite terminals of ADAM10-cKO cortical neurons (*C*). Quantification of branches and comets was shown at the right (*D*). (*E*, *F*) Western blot analysis of acetylated tubulin protein levels in Adam10-knockdown (*E*) or -knockout (*F*) cortical neurons. Quantification of the relative change of Ac-Tub in cKO neurons was shown at the right. (*G*) Immunofluorescent staining of acetylated tubulin (Ac-tub) in cultured early cortical neurons electroporated with *scramble* (sh.scr) and *Adam10* shRNA (sh.AD10) construct. (*H*, *I*) MT structural change shown by higher resolution microscope image. The MT structure was immunostained with anti-Ac-Tub antibody and shown as Z-projected images (red) or Neurolucida-traced architecture (pink). (*I*) The total MT fiber length (left) and number (right) were quantitatively analyzed using Neurolucida-traced MT structure ($n = 19$, con; $n = 22$, Dcx-Cre). Scale bar, 50 μm in (*A*); 5 μm (*C*) (*H*), 20 μm (*G*).

imply that ADAM10 may control the dynamics of MT cytoskeleton to regulate radial migration.

The polarized distribution of acetylated tubulin along the leading process and soma regulates MT dynamics (Creppe et al. 2009; Wloga and Gaertig 2010). To examine the effect of ADAM10 on MT structure, we examined the change of acetylated α-tubulin (Ac-Tub) in cultured early cortical neurons. Interestingly, ADAM10 knockdown (sh.AD10) in early neurons caused a significant decrease in Ac-Tub relative to total α-tubulin (Fig. 4*E*). Conditional knockout of ADAM10 in cortical neurons produced similar reduction of Ac-Tub (Fig. 4*F*). Moreover, immunostaining analysis showed that the polarized distribution of Ac-Tub in newborn neurons was also disrupted (Fig. 4*G*). In WT neurons, Ac-Tub was enriched in the distal neurites or leading processes, whereas in ADAM10-deficient neurons, ac-Tub staining was dramatically diminished in the distal end of neurites.

The MT structure is a critical component of the migration machinery, and plays an essential role in dragging the movement of neuron soma (Tsai and Gleeson 2005; Yokota et al. 2009; Eom et al. 2011). To examine whether ADAM10 knockout caused any disruption of MT structure in neuron soma, we took super-resolution light microscope images of the MT structure of cultured cortical neurons (Fig. 4*H*). The MT bundles in control neurons form a bag, and the MT bundles surrounding cell nuclear show strong signals of Ac-Tub (Fig. 4*H*, left). However, the MT bundles encasing cell nuclear in Adam10-cKO neurons are relatively broken down, and the signal of Ac-Tub was significantly reduced (Fig. 4*H*, right). Quantification of Neurolucida-traced MT structure showed that the total length of soma MT structure was significantly downregulated by around 52% (Fig. 4*I*, left), and the MT fiber numbers were also reduced by ~38% (Fig. 4*I*, right). Taken together, these findings suggest that ADAM10 modulates MT dynamics and affects cell motility to control radial migration.

ADAM10/Notch Signaling Regulates DCX Expression

In the above study, we identified Notch as a critical mediator of ADAM10-triggered RIP to regulate cortical radial migration, whereas how NICD regulates radial migration remains elusive. Given that NICD regulates the transcription of target genes thereby inhibiting neuronal specification in proliferating neural

stem cells (Yoon and Gaiano 2005), it is highly possible that NICD targets distinct downstream genes in early neurons to control their migration. Interestingly, pharmacological intervention of Notch in neuron culture (Ferrari-Toninelli et al. 2008), and the identification of *short stop* gene (a cytoskeleton linker protein) as a target of NICD in *Drosophila* foregut (Fuss et al. 2004), both link Notch to the MT cytoskeleton, implying that ADAM10/Notch signaling may regulate the migration of newborn cortical neurons through cytoskeleton modulators in mammals. Since MAPs regulate MT dynamics and affect the posttranslational modifications of tubulin (Heng et al. 2010; Wloga and Gaertig 2010), the reduction of Ac-Tub seen in ADAM10-deficient neurons suggested that ADAM10-dependent RIP of Notch may affect the protein levels of certain MAPs.

To examine this possibility, protein levels of a cohort of MAPs were studied. We found that DCX, Tau1, and MAP2, but not LC3B or MAP1B, were significantly downregulated in ADAM10-knockdown (sh.AD10) cortical neurons (Fig. 5*A*,*B*). Furthermore, we examined the change of mRNA levels in cultured cortical neurons. Surprisingly, only the mRNA level of *Dcx* was significantly downregulated, whereas the mRNA levels of *Map2* and *Tau1* were unchanged (Fig. 5*C*). As the protein level of MAP2 and Tau1 was found to be reduced in *Dcx* KO mice (Fu et al. 2013), it is reasonable that the reduction of MAP2 and Tau1 in ADAM10-knockdown neurons was secondary to the decreased expression of *Dcx* mRNA. Consistently, knockdown of Notch1 also reduced the levels of *Dcx* mRNA (Fig. 5*F*). We further observed by western blot that DCX protein levels were decreased in ADAM10-cKO or Notch1 knockdown cortical tissue (Fig. 5*D*,*E*). Moreover, immunofluorescent staining of DCX also showed that its levels were significantly reduced in ADAM10-deficient neurons in CP (Fig. 5*H*). Taken together, these results indicate that ADAM10-initiated RIP of Notch controls the expression of DCX.

Since both Notch1 and Notch2 are required for neuron migration (Hashimoto-Torii et al. 2008), we also examined the effect of notch2 knockdown on the change of DCX expression. We found no significant change on DCX protein level in notch2-KD neurons (Fig. 5*G*), possibly due to about 4 times higher expression level of Notch1 mRNA compared with notch2, which is consistent with the difference in the phenotype that migration defect in notch1 KO mice is significantly more apparent than that in notch2 KO mice (Hashimoto-Torii et al. 2008).

Dcx is a Direct Transcriptional Target of the NICD/RBPJ Complex

Released NICD translocates into cell nucleus and forms a transcription activation complex with RBPJ, which binds to the consensus RBPJ binding sequence (RBS) "NNTGGGAA" (Kopan and Ilagan 2009). Whether the regulation of *Dcx* expression by ADAM10/Notch signaling depends on transcription requires further investigation. We discovered 2 RBS motifs in the promoter sequence of the mouse *Dcx* (Fig. 6*A*). Using ChIP analysis, we found that both NICD and RBPJ proteins were enriched in the RBS loci of mouse *Dcx* (Fig. 6*B*), indicating that the NICD/RBPJ complex binds directly to the promoter of *Dcx* gene.

We tested whether the putative NICD/RBPJ motifs in the *Dcx* promoter are functional by performing luciferase assays on cultured cortical neurons. DNA fragment of 2.7 kb wt or mutant

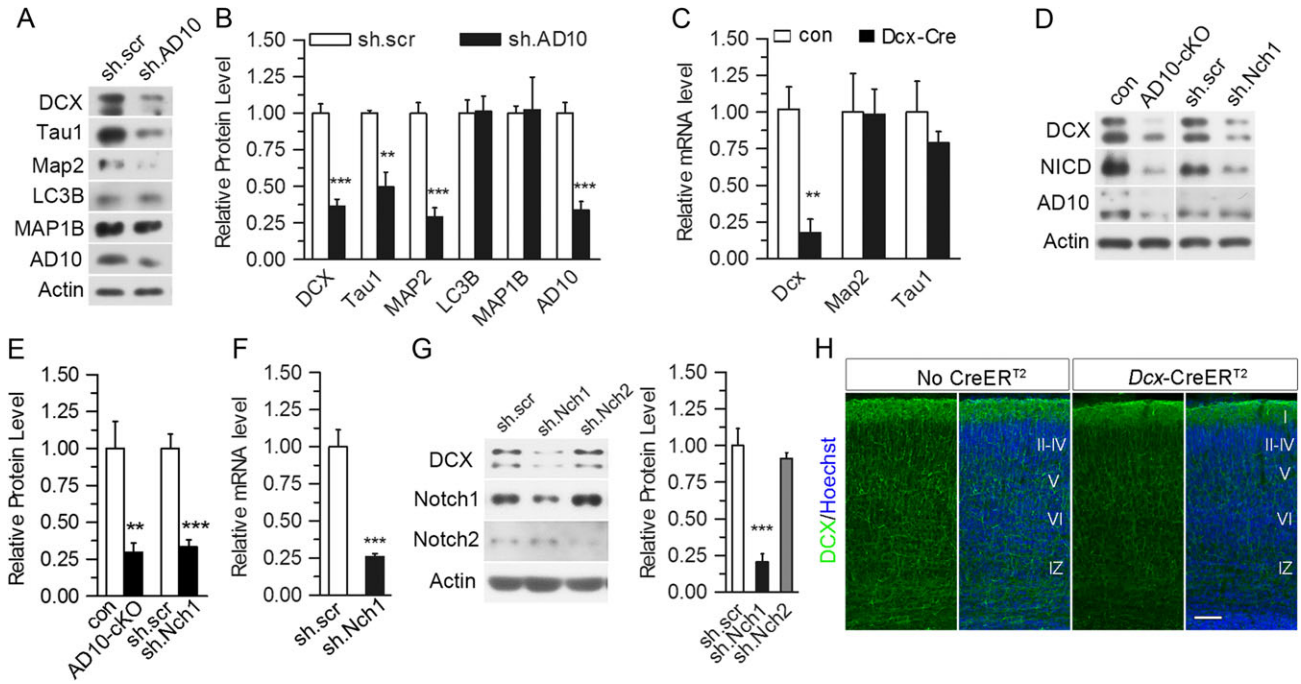

Figure 5. ADAM10/Notch signaling regulates the expression of MAPs. (*A*, *B*) Knockdown of ADAM10 led to reduced protein level of several MAPs in cortical neurons cultured from E18 embryos shown by western blot. The change of relative protein levels was statistically analyzed in (*B*). (*C*) Knockout of ADAM10 downregulated the mRNA expression levels of *Dcx*, but leaving that of *Map2* and *Tau1* unchanged. To knockout *Adam10*, cortical neurons from E18 *Adam10*$^{f/f}$ embryos were electroporated with pDcx-Cre plasmid and then cultured for 48 h before RNA extraction. (*D*, *E*) Knockout of ADAM10 or knockdown of Notch1 leads to reduced expression level of DCX protein. Cortical neurons from E18 *Adam10*$^{f/f}$ embryos were electroporated with pDcx-Cre, sh.Nch1, or control plasmid and then cultured for 48 h for western blot. (*F*) Knockdown of *Notch1* (sh.Nch1) downregulated the mRNA expression levels of *Dcx*. (*G*) Knockdown of Notch1 but not Notch2 significantly reduced the expression level of DCX protein. Quantification shown at the right. (*H*) Immunofluorescent staining of DCX expression in cerebral cortex of brain slices from E19.5 control and *Dcx*-CreER^{T2}:*Adam10*$^{f/f}$ embryos induced with tamoxifen. Scale bar, 50 μm.

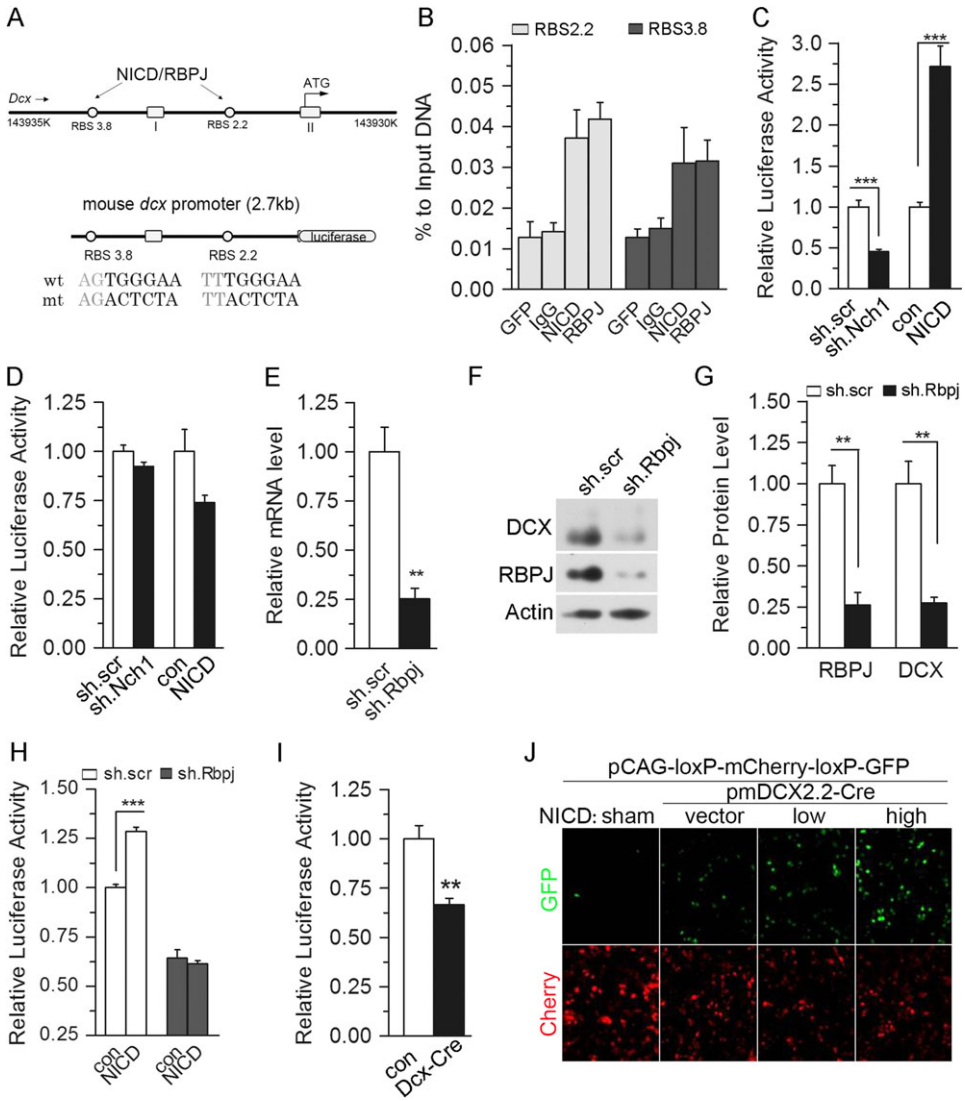

Figure 6. *Dcx* is transcriptionally controlled by the NICD/RBPJ complex. (*A*) Top, Localization of putative NICD/RBPJ binding sequences in the mouse *Dcx* gene. Binding motifs are marked with a hollow circle and exons are shown as rectangles. Bottom, design of *Dcx* promoter luciferase constructs based on pGL3-basic plasmid. The sequence of wt and mutated RBPJ binding sequences is shown below marked positions. (*B*) ChIP analysis showing the enrichment of NICD and RBPJ in the RBS loci within the *Dcx* promoter region. (*C, D*) Luciferase assay of *Dcx* promoter with normal (*C*) and mutated (*D*) RBPJ binding sequences. Neurons co-electroporated with luciferase constructs and *Notch1* shRNA (sh.Nch1) or NICD expression plasmid, and analyzed 48 h later. (*E, F, G*) Knockdown of *Rbpj* (sh.Rbpj) led to reduced level of *Dcx* mRNA (*D*) and protein (*F*) in cultured early cortical neurons. Quantification of protein change was shown at the right of (*G*). (*H*) Regulation of *Dcx* promoter by Notch is RBPJ dependent. Knockdown of Rbpj (sh.Rbpj) led to insensitive of Dcx promoter to the overexpression of NICD. (*I*) Luciferase assay of the *Dcx* promoter in *Adam10*-deficient cortical neurons. Neurons were electroporated with *Dcx*-Cre construct to knockout *Adam10* and analyzed 48 h later. (*J*) Dual-fluorescence reporter assay of *Dcx* promoter activity. The *Dcx*-Cre plasmid contains a RBS2.2 binding sequence and reporter construct pCAG-loxp-mCherry-loxp-GFP was transfected into 293T cells together with different doses of NICD plasmid. The number of GFP^+ cells is increased in the presence of NICD in a dose-dependent manner.

Dcx promoter was cloned and inserted into p*GL3-basic* luciferase construct (Fig. 6A). First, in neurons transfected with the wt *mDcx2.7* construct, the luciferase activity showed a more than 50% reduction when Notch1 was knocked down, while the luciferase activity of neurons cotransfected with NICD was dramatically upregulated (Fig. 6C). In neurons transfected with the mutant *mDcx2.7* construct (mt.dcx), the luciferase activity was not affected by either knockdown of Notch1 or overexpression of NICD (Fig. 6D), suggesting that Notch regulates the transcriptional activity of the *Dcx* promoter.

To examine whether RBPJ is required for the regulation of DCX expression, we knocked down RBPJ in cortical neuron by *Rbpj* targeting shRNA (sh.Rbpj). DCX was significantly downregulated at both mRNA level (Fig. 6E) and protein level (Fig. 6F,G). Moreover, the luciferase activity of wt mDcx2.7 promoter was downregulated when *Rbpj* was knocked down. Furthermore, the upregulation of pGL3-mDcx2.7 luciferase activity by overexpressed NICD was abolished in the absence of RBPJ (Fig. 6H), demonstrating that RBPJ is required for the transcriptional regulation of *Dcx* promoter. Consistently, the luciferase activity of wt mDcx2.7 promoter was also downregulated in ADAM10-cKO neurons (Fig. 6I). Moreover, we cotransfected p*Dcx-Cre* (containing the RBS2.2 motif) and reporter construct p*CAG-LSL-mCherry-GFP* into 293T cells, and observed an increase in the number of GFP^+ cells in NICD transfected wells in a dose-dependent manner (Fig. 6J), demonstrating again that the transcriptional activity of the *Dcx* promoter is regulated by NICD. Finally, we cloned human *DCX* promoter to pGL3-basic construct and found similar

downregulation of luciferase activity when *ADAM10* (*sh.AD10*) or *NOTCH1* (*sh.Nch1*) was knocked down (Supplementary Fig. 3). Taken together, these results demonstrate that the NICD/RBPJ complex regulates the transcription of targeted MAP gene *Dcx*.

DCX Rescues the Migration Defect of ADAM10-cKO Neurons

In the above experiments, we demonstrated that the expression of MAP proteins was reduced in ADAM10-deficient neurons. However, it was unclear whether the regulated expression of MAP proteins underlies the function of ADAM10 in cortical radial migration. To examine this, we expressed NICD into ADAM10-deficient neurons. Re-expression of NICD reversed the decrease of *Dcx* mRNA (Fig. 7*A*) and DCX protein (Fig. 7*B*,*C*). Moreover, expressing either NICD or DCX significantly reversed the downregulated level of Ac-Tub caused by ADAM10 knockout (Fig. 7*B*,*C*), indicating that NICD-induced DCX expression may mitigated the impaired MT structure in ADAM10-cKO neurons. Indeed, restoring NICD or DCX largely rescued the impaired neuronal motility of ADAM10-deficient neurons shown by transwell assay (Fig. 7*D*,*E*), suggest that NICD or DCX could functionally restore the disrupted cell motility of ADAM10-deficient neurons. Moreover, overexpression of either NICD or DCX in ADAM10-deficient neurons apparently rescued the MT structure in soma region (Fig. 7*F*,*G*), further demonstrating DCX as a critical effector downstream of ADAM10/Notch signaling to modulate MT structure.

As DCX-regulated MT dynamic plays a critical role in radial migration (Schaar et al. 2004; Tanaka et al. 2004a, 2004b; Deuel et al. 2006), we then test whether DCX rescues the migration defect of ADAM10-deficient neurons. We found replenished DCX largely mitigated the migration defects of ADAM10-cKO neurons (Fig. 7*H*). Overexpression of DCX in ADAM10-cKO neurons led to an ~80% rescue of their final positioning in cortical layers II–IV, suggesting that DCX is a critical effector in ADAM10/Notch signaling to regulate cortical radial migration.

Discussion

In this study, we report that ADAM10 plays a direct role in the radial migration of newborn cortical neurons, primarily via initiating the RIP of Notch to release NICD. Further study shows that ADAM10/Notch signaling controls neuron migration through the affection of neuron motility and modulation of MT stability. Specifically, we identified DCX as a direct target of the NICD/RBPJ transcriptional activation complex in the regulation of MT dynamics. Moreover, overexpressing DCX significantly mitigated the migration defects in ADAM10-cKO neurons. These results suggest ADAM10-initiated RIP of Notch as a critical membrane signal mechanism to regulate neuron migration.

ADAM10 is highly expressed in both neural progenitor cells and newborn neurons in the developing cerebral cortex. The migration defect caused by Nestin-Cre–mediated removal of ADAM10 may partly result from the depletion of neural progenitors and their precocious differentiation (Jorissen et al. 2010). To dissect the functional role of ADAM10 specifically in newborn cortical neurons, we carried out neuron-specific deletion or knockdown of *Adam10* through Cre recombinase driven by *Dcx* promoter (Franco et al. 2011). The observed migration defects demonstrate that ADAM10 is directly required for cortical radial migration. Moreover, the protease activity is essential for the function of ADAM10 in radial migration, whereas the interaction of ADAM10 with other membrane-associated partners may help to determine the substrate specificity and cleavage efficiency (Murphy 2008; Endres and Fahrenholz 2012). Therefore, the direct requirement of ADAM10 in radial migration not only uncovers a novel function of ADAM10 during neuronal development, but also reveals ADAM10-initiated RIP as a new membrane signal transduction paradigm in the regulation of neuron migration (Ayala et al. 2007; Cooper 2014). Together with paradigms such as ligand-receptor binding or interaction between adhesion molecules, ADAM10-initiated RIP enables newborn neurons with multiple strategies to sense environmental stimuli and precisely orchestrate intracellular molecular machinery for migration.

We identify Notch as a critical downstream mediator selectively underlying the requirement of ADAM10-triggered RIP in cortical radial migration. This is consistent with the functional correlation that ADAM10-deficient mice highly resemble Notch knockout mice in the malformation of brain structure (Yoon and Gaiano 2005; Jorissen et al. 2010). We found that ADAM10-initiated RIP of Notch is the major source to generate NICD in neurons in vivo, confirming it as a conserved signal from neural progenitor cells. Although many other ADAM10 substrates and membrane-associated partners, such as APP, *N*-cadherin, Ephrin, and integrin isotypes, have been linked to migration (Cooper 2014), and particularly APP shows many similarities to Notch, including the interaction with Dab1, cleavage by ADAM10, and nuclear translocation of the intracellular domain (Hoe et al. 2006; Young-Pearse et al. 2007; Hashimoto-Torii et al. 2008), we found only replenished NICD into ADAM10-deficient neurons obviously mitigated their migration defects. This is also supported by the previous report that Notch is critically involved in cortical radial migration (Hashimoto-Torii et al. 2008). However, as ADAM10-triggered RIP not only releases intracellular fragments of its substrates, but also cut cell–cell adhesion molecules and membrane-associated ligands (Murphy 2008; Weber and Saftig 2012), we could not rule out the potential involvement of other ADAM10 downstream mechanism in the regulation of neuron migration.

A number of recent studies have demonstrated diversified roles of Notch in postmitotic neurons (Ables et al. 2011), particularly in the regulation of dendritogenesis and synaptic plasticity (Jorissen et al. 2010; Pierfelice et al. 2011). However, how NICD regulates neuron migration remains a critical question. We found that neuron cell motility decreased, and the level of acetylated tubulin was reduced and its polarized distribution was disrupted in the absence of ADAM10. This leads to the finding that ADAM10-initiated RIP of Notch regulates the expression levels of a cohort of MAPs, thus raising a conceptually new hypothesis that ADAM10/Notch signaling regulates the expression of MAPs to control MT cytoskeleton dynamics and neuronal motility. Although it is distinct from the well-known roles of Notch in proliferation and fate control (Yoon and Gaiano 2005), this mechanism is supported by several recent findings, such as Notch inhibitor Numb regulates the movement of centrosome (organizer of MT structure) (Jauffred et al. 2013), and activation of Notch by its ligand Jagged1 leads to increased MT stability in culture cortical neurons (Ferrari-Toninelli et al. 2008). There are accumulating reports that Notch signaling underlies cellular events with morphological remodeling and cell movement, such as cell division, dendritic branching of neurons, and metastasis of cancer cells (Pierfelice et al. 2011; Ntziachristos et al. 2014). Our finding that Notch regulates the expression of MAPs to control MT stability provides a direct link how Notch is involved in these cellular processes.

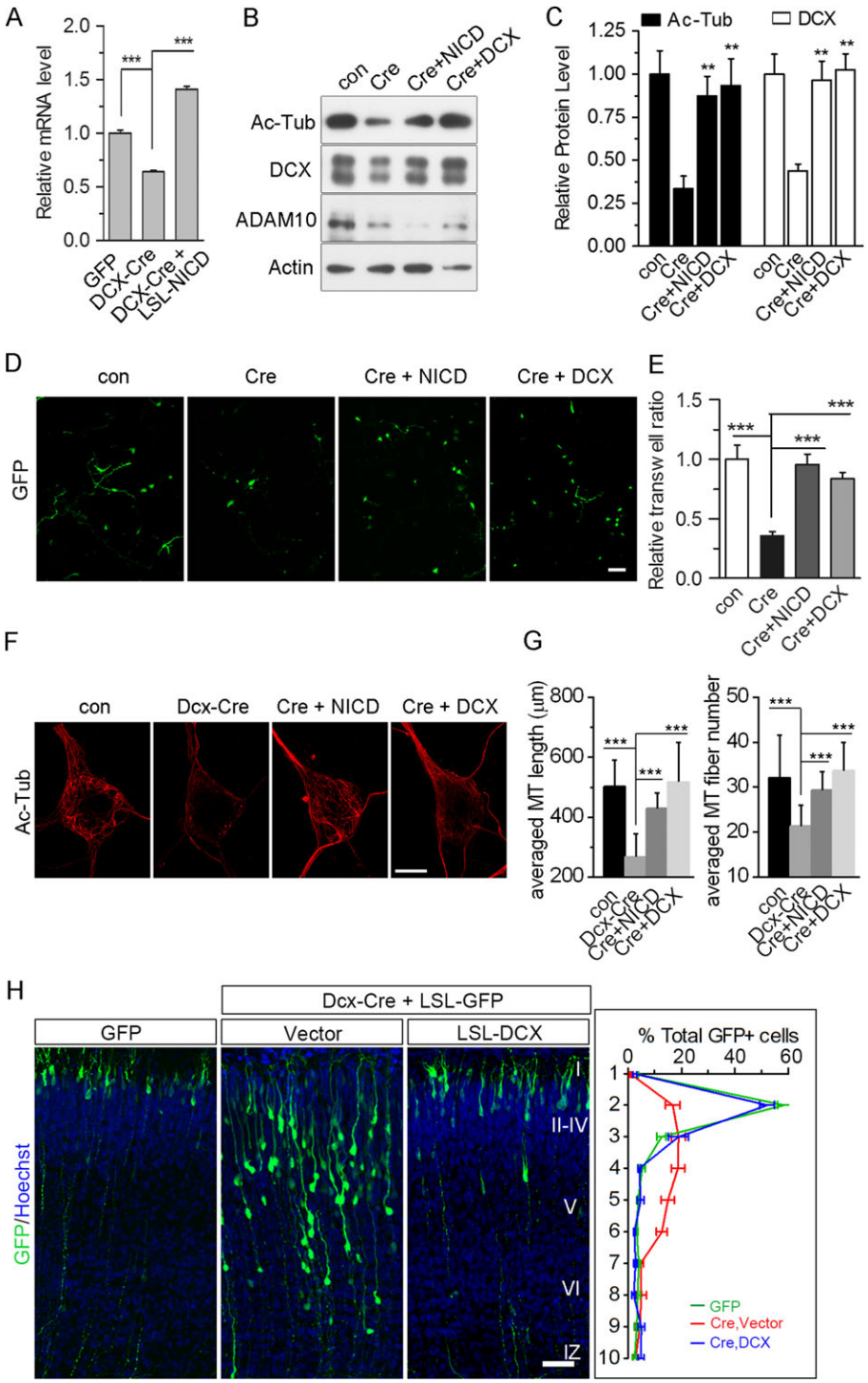

Figure 7. DCX rescues the impaired MT structure and neuron migration in ADAM10-deficient cortical neurons. (*A*) ADAM10 deficiency downregulated DCX mRNA expression is rescued by the overexpression of NICD. (*B*, *C*) Downregulated Ac-Tub level in ADAM10-deficient cortical neurons was rescued by overexpression of either NICD or DCX. Quantification of the rescue effect was shown in (*C*). (*D*, *E*) Replenished NICD or DCX rescued defect of neuron motility in ADAM10-cKO cortical neurons. Statistical analysis is shown in (*E*). (*F*, *G*) Replenished NICD or DCX rescued the disrupted MT structure in ADAM10-cKO cortical neurons. MT structure was visualized by anti-Ac-Tub antibody and imaged with higher resolution microscope image. (*G*) Quantification of the total length (left) and number (right) of MT fibers was analyzed using Neurolucida-traced MT structure ($n = 23$, con; $n = 20$, Dcx-Cre; $n = 19$, Cre + NICD; $n = 21$, Cre + DCX). (*H*) Replenished DCX mitigated migration defects in ADAM10-cKO cortical neurons. Control and *Adam10*$^{f/f}$ embryos were electroporated with pDcx-Cre, pCAG-LSL-GFP, and pCAG-LSL-DCX at E14.5 and sacrificed 4 days later. Quantitative analysis of cell distribution is shown at the right ($n = 5$ for each group). Scale bar, 50 μm in (*D*), (*H*), 5 μm in (*F*).

Antimitotic or MT compounds have been shown with promising therapeutic effect to treat leukemia, which is associated with mutations of Notch1 in human patients (Ntziachristos et al. 2014). The link between ADAM10-initiated RIP of Notch and MT cytoskeleton should shed light on the development of new drugs targeting related diseases.

In mouse cerebral cortex, neuron migration takes several days to reach the superficial layer, making both the long-term transcriptional regulation and short-term posttranslational modification of MAPs essential to the orchestrated migration machinery (Schaar et al. 2004; Tanaka et al. 2004b; Kwan et al. 2012). We identified *Dcx* as a novel transcriptional regulation target of NICD. As DCX plays important roles in regulating MT dynamics and neuron migration (Gleeson et al. 1999; Bai et al. 2003; Deuel et al. 2006), this finding demonstrates that transcriptional regulation of MAP expression by NICD is critical in the control of neuron migration. Additionally, *Dcx* is found to be transcriptionally repressed by SnoN1-FOXO1 complex to regulate axonal branching and radial migration of granule cells in cerebellum (Huynh et al. 2011), suggesting that transcriptional regulation of *Dcx* may serve as a critical pathway to control neuron migration. However, it should be mentioned that the impaired MT dynamics caused by ADAM10-initiated RIP of Notch may depend on changing the expression of a group of MAPs, not solely DCX. Full characterization of the target genes of ADAM10-initiated RIP of Notch in radial migration requires further study, especially whole-genome expression analysis using single cell sequencing. Considering that only mild migration and lamination defect is observed in *Dcx* knockout mouse brain (Deuel et al. 2006), whereas smooth cortex and abnormal double cortices occur in *DCX* mutant human brain (Berg et al. 1998), the functional consequence of DCX deficiency on cortical neuron migration and lamination may depends on other factors, such as the thickness of CP which possibly defines short or long distances and thereby differential modes of neuron migration in mouse and human brain (Lui et al. 2011). Nevertheless, replenished DCX largely mitigated migration defect caused by ADAM10 knockout, indicating that DCX expression can largely recover ADAM10 deficiency impaired MT structure, as supported by the result of transwell assay. Therefore, DCX may not be indispensable in the control of migration, but is sufficient for the recovery of MT structure and the rescue of migration defect of ADAM10-deficient neurons in mouse cerebral cortex.

Supplementary Material

Supplementary material are available at *Cerebral Cortex* online.

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Notes

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Author contributions

X.W.C., Z.Y., and Z.-Q.X. designed the experiments; X.W.C., Z.Y., P.-F.L., and R.-C.C. carried out the experiments and analyzed data; X.H.W. provided the *adam10*$^{f/f}$ mice; J.W., S.R.W., and Y.S. provided the experimental assistance for electroporation, cell culture, and genotyping; X.W.C. prepared the manuscript; Z.-Q.X. supervised the work.

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