Regulation of Radial Glial Survival by Signals from the Meninges

Randor Radakovits,¹ Claudia S. Barros,¹ Richard Belvindrah,¹ Bruce Patton,² and Ulrich Müller¹

¹The Scripps Research Institute, Department of Cell Biology, Institute for Childhood and Neglected Disease, La Jolla, California 92037, and ²Oregon Health & Science University, Portland, Oregon 97239

Radial glial cells (RGCs) in the developing cerebral cortex are progenitors for neurons and glia, and their processes serve as guideposts for migrating neurons. So far, it has remained unclear whether RGC processes also control the function of RGCs more directly. Here, we show that RGC numbers and cortical size are reduced in mice lacking $\beta 1$ integrins in RGCs. TUNEL stainings and time-lapse video recordings demonstrate that $\beta 1$ -deficient RGCs processes detach from the meningeal basement membrane (BM) followed by apoptotic death of RGCs. Apoptosis is also induced by surgical removal of the meninges. Finally, mice lacking the BM components laminin $\alpha 2$ and $\alpha 4$ show defects in the attachment of RGC processes at the meninges, a reduction in cortical size, and enhanced apoptosis of RGC cells. Our findings demonstrate that attachment of RGC processes at the meninges is important for RGC survival and the control of cortical size.

Introduction

Cortical neurons and glia are generated from radial glial cells (RGCs) in the ventricular neuroepithelium. During early stages of cortical development, RGCs divide predominantly symmetrically, generating pairs of RGCs and expanding the surface area of the developing cortex in the lateral and longitudinal dimensions. As cortical development progresses, asymmetric cell divisions become prominent, generating two distinct RGC daughters. One daughter remains a RGC, while the other becomes an intermediate progenitor or a neuron. At this stage, the number of neurons and intermediate progenitors that are produced by each RGC will determine the thickness of the cortex. At late stages of corticogenesis, RGCs finally generate astrocytes (Fishell and Kriegstein, 2003; Götz and Huttner, 2005).

Signaling molecules that control the differentiation and survival of RGCs, including ligands for Notch and Eph receptors, are expressed in RGCs, intermediate progenitors, and early generated neurons, suggesting that short-range interactions in the ventricular neuroepithelium regulate RGC numbers (Gaiano et al., 2000; Depaepe et al., 2005; Yoon et al., 2008). RGCs also extend radial processes that are anchored at the meningeal basement membrane (BM). The meninges consist of three distinct mesenchymal cell layers. The inner-most layer, named pia, produces the BM covering the cortex and serves as the source for blood vessels in the superficial region of the cerebral cortex. The middle layer, named arachnoid, has

important function in the resorption of CSF, whereas the outermost layer, the dura, is a collagenous structure that is tightly attached to the calvarium (McLone and Bondareff, 1975; Bauer et al., 1993; Sievers et al., 1994). *In vitro* studies have provided evidence that RGC processes might receive signals from cells in the meninges and the adjacent Cajal-Retzius (CR) cell layer. For example, meningeal cells stimulate cell proliferation and neuronal differentiation (Barakat et al., 1981; Gensburger et al., 1986), whereas transplantation of CR cells from embryonic mice into adult cerebella rejuvenates Bergmann glia into a RGC phenotype (Soriano et al., 1997). Furthermore, reelin, which is expressed in CR cells, affects the expression of brain lipid binding protein in RGCs (Hartfuss et al., 2003). Finally, a diffusible signal from the embryonic forebrain regulate RGC differentiation (Hunter and Hatten, 1995).

In vivo evidence that supports a role for RGC processes in the control of RGC differentiation is lacking. Mutations and chemical perturbations that disrupt attachment of RGC processes at the meningeal BM lead to process retraction (Pehlemann et al., 1985; Sievers et al., 1985, 1986; von Knebel Doeberitz et al., 1986; Hartmann et al., 1992; Georges-Labouesse et al., 1998; Miner et al., 1998; Costell et al., 1999; De Arcangelis et al., 1999; Supèr et al., 2000; Graus-Porta et al., 2001; Halfter et al., 2002; Michele et al., 2002; Moore et al., 2002; Beggs et al., 2003; Poschl et al., 2004; Niewmierzyska et al., 2005), but the extent to which these perturbations affect RGC generation and survival has remained unclear. One study has reported that mutations in the meningeal BM component perlecan affect cortical progenitor proliferation, but a second study has failed to find such defects (Haubst et al., 2006; Girós et al., 2007).

To determine whether attachment of RGC processes at the meningeal BM is important to regulate RGC generation and survival, we have analyzed cortical development in *Itgb1-CNSko* mice. These mice lack $\beta 1$ integrins in RGCs, leading to

Received Nov. 18, 2008; revised May 11, 2009; accepted May 13, 2009.

This work was funded by National Institutes of Health Grants NS046456 and MH078833 (U.M.), a training grant from the California Institute of Regenerative Medicine (R.B.), and a Christopher Reeve Foundation fellowship (C.S.B.). We thank members of our laboratory for critical comments on this manuscript.

Correspondence should be addressed to Ulrich Müller, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037. E-mail: umueller@scripps.edu.

DOI:10.1523/JNEUROSCI.5537-08.2009

Copyright © 2009 Society for Neuroscience 0270-6474/09/297694-12\$15.00/0

the detachment of their radial processes from the meninges (Graus-Porta et al., 2001). Here, we demonstrate that *Itgb1-CNSko* mice have a significantly smaller brain compared with control mice, and we show that the reduction in brain size is at least in part a consequence of RGC death that is caused by detachment of RGC processes from the meningeal BM. Our findings suggest that the radial processes of RGCs, which have well established roles in the guidance of neuronal migration, are also important to receive contact-mediated and/or diffusible signals that could be derived from several sources in the meninges including meningeal fibroblasts, endothelial cells, the CSF, and the blood stream.

Materials and Methods

Animals. *Itgb1-CNSko* mice were generated by crossing *Itgb1^{fllox/flox}* mice with *Itgb1^{fllox/+}*, *nestin-CRE^{+/-}* mice (Graus-Porta et al., 2001). The laminin $\alpha 2$ and laminin $\alpha 4$ mutants (LNa2/a4-KO) have been described previously (Sunada et al., 1994; Xu et al., 1994; Patton et al., 2001). As controls we used littermates carrying floxed alleles but lacking CRE-recombinase, or mice expressing CRE but lacking floxed alleles because they were indistinguishable from wild-type mice.

Histology and immunohistochemistry. Nissl staining and the analysis of histological sections by immunofluorescence was performed as described previously (Graus-Porta et al., 2001). Antibodies were as follows: Tbr2 rabbit polyclonal (Millipore Bioscience Research Reagents), Pax6 rabbit polyclonal (Covance Research Products), Cux1 rabbit polyclonal antibody against CDP (Santa Cruz Biotechnology), Tbr1 rabbit polyclonal (Millipore Bioscience Research Reagents), Sox2 rabbit polyclonal (Millipore Bioscience Research Reagents), bromodeoxyuridine (BrdU) mouse IgG (BD Biosciences), Ki67 rabbit polyclonal (Novocastra), Cleaved Caspase-3 (Asp175) rabbit polyclonal (Cell Signaling Technology), Laminin rabbit polyclonal (kindly provided by L. Sorokin, University of Münster, Münster, Germany), RC2 mouse IgM (Hybridoma Bank), Reelin mouse IgG (Millipore Bioscience Research Reagents), Phospho-histone H3 (Ser 10; Upstate). Sections were mounted with ProLong Gold antifade mounting medium (Invitrogen). Stainings were analyzed by confocal microscopy (Fluoview-LSM; Olympus Optical) or deconvolution microscopy (Deltavision; Applied Precision). For BrdU labeling, pregnant females were injected intraperitoneally with 100 mg BrdU/kg body weight (10 mg/ml BrdU in PBS). Apoptosis was analyzed by staining for activated Caspase-3 and by the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) using the ApopTag Red In Situ Apoptosis Detection Kit (Millipore Bioscience Research Reagents). Quantifications were performed on 12- μ m-thick coronal sections at rostral and caudal levels. The labeling index was calculated by determining the percentage of BrdU-labeled cells that were also Ki67 positive, and the quitting fraction was calculated by determining the percentage of BrdU-labeled cells that are not positive for Ki67. The dividing fraction of all RGCs was calculated by determining the percentage of Pax6-positive cells that are also Ki67 positive. All values given are mean $\pm$ SD and a Student's *t* test was performed to evaluate statistical significance.

Analysis of the size of the cerebral cortex. All size measurements were performed on three mutant and three control littermates using MetaMorph software (Molecular Devices). Brains were fixed overnight at 4°C in 4% PFA, and serial 50 μ m Vibratome sections (coronal for width measurements, sagittal for length measurements) were prepared and stained with Nissl as described previously (Graus-Porta et al., 2001). Three consecutive sections each were quantified at three different histological levels. The thickness of the cerebral cortex was measured at similar anatomical levels in three consecutive sections. All values given are mean $\pm$ SD and a Student's *t* test was performed.

Electroporation and slice cultures. Mouse brains were electroporated and subsequently cultured as slices as previously described (Polleux et al., 2002; Hand et al., 2005). Time-lapse imaging was performed using an Olympus IX70 microscope (Olympus Optical) with a humidified temperature-controlled chamber supplied with oxygen and CO₂. Im-

aging of slices started 12–16 h after electroporation and images were captured every 15 min for up to 96 h; each frame in the time-lapse videos was generated from an 8- μ m-thick z-stack containing nine separate images that were compressed into a single frame. For time-lapse microscopy we used the pCIG2 vector (Hand et al., 2005), which expresses enhanced green fluorescent protein (EGFP) under the control of a cytomegalovirus-enhancer/chicken β -actin promoter. To inactivate integrin $\beta 1$ in slice cultures from *Itgb1^{fllox/flox}* animals, we used a pCIG2 construct containing CRE-internal ribosomal entry site (IRES)-EGFP.

Results

Microcephaly in *Itgb1-CNSko* mice

We have previously demonstrated that RGC processes are detached from the meningeal BM in *Itgb1-CNSko* mice, in which a floxed integrin $\beta 1$ subunit gene (*Itgb1^{fllox}*) has been inactivated in RGCs using *nestin-CRE* (Graus-Porta et al., 2001). Analysis of the size and weight of *Itgb1-CNSko* mice and their organs revealed no differences to wild-type controls (supplemental Fig. 1B–D, available at www.jneurosci.org as supplemental material), with the exception of the brain, which was significantly smaller in the mutants (Fig. 1A,B; supplemental Fig. 1A, available at www.jneurosci.org as supplemental material). Quantifications of cortical size demonstrated that its length along the rostrocaudal axis was reduced in postnatal day (P) 21 animals by $17 \pm 1\%$ (Fig. 1C,D,I). A similar size decrease was observed in the lateral extension of the cortex (Fig. 1J). The size reduction was already detectable by P0 (Fig. 1E,F; supplemental Fig. 1A, available at www.jneurosci.org as supplemental material), but the telencephalic vesicles at embryonic day (E) 11 were not affected (Fig. 1G,H).

We next measured the thickness of the cortical wall in P21 animals. Although cortical cell layers in *Itgb1-CNSko* mice meander because neurons invade the cortical marginal zone in areas where the meningeal BM is disrupted (Graus-Porta et al., 2001), the overall thickness of the cortical wall was not altered (Fig. 1K,L,Q). To confirm these findings at higher resolution and to test whether the number of neuronal subtypes within cortical layers might be changed, we stained histological sections with antibodies to Tbr1 and Cux1. Tbr1 is expressed in subpopulations of neurons in layers II/III and VI, and Cux1 in subpopulations of neurons in layers II–IV. We observed no difference in the number of Tbr1- and Cux1-positive neurons between wild-type and mutant animals (Fig. 1M–P,R,S). The specific defect in cortical surface area without a change in cortical layers suggests that in *Itgb1-CNSko* the expansion of the neural precursor pool is affected, but not their competence to differentiate into neuronal subtypes.

Loss of Pax6-positive neural precursors

Perturbations in the growth of the surface area of the cerebral cortex could be caused by defects the generation or maintenance of RGCs. We therefore quantified the number of Pax6-positive RGCs at different developmental ages. As reported earlier, the number of Pax6-positive cells declined in wild-type mice between E11 and E18 (Fig. 2A–I). However, the decline was much faster in *Itgb1-CNSko* mice. Whereas the number of neural precursors in wild-types and mutants was similar at E11 (Fig. 2A,B,I), a $22 \pm 3.2\%$ reduction was observed by E13 (Fig. 2C,D,I), and a $47 \pm 5.8\%$ loss by E18 (Fig. 2G–I). In coronal sections, a loss of similar magnitude was observed at all levels along the rostrocaudal axis of the ventricular neuroepithelium (data not shown), indicating that all functional subdomains of the cerebral cortex were similarly affected. Quantification of the number of Tbr2-positive basal progenitors, which are generated from Pax6-positive RGCs,

Figure 1. Defects in the size of the cerebral cortex in *Itgb1-CNSko* mice. **A, B**, Whole mounts of a cortical hemisphere from a wild-type (wt) and *Itgb1-CNSko* mouse at P21. The red arrows are the same size in **A** and **B** and highlight the difference in cortical size. **C, D**, Nissl-stained sagittal sections of P21 brains. The red arrows are the same size in **C** and **D** and highlight the difference in cortical length. **E–H**, Nissl-stained coronal sections from P0 and E11 brains. **I, J**, Quantification of the length and width of the cerebral cortex at three anatomical levels demonstrates that the cortex in *Itgb1-CNSko* mice is significantly shorter ($p = 0.0029$, 0.0174 , and 0.0047 ; $n = 3$) and narrower ($p = 0.0045$, 0.0007 , and 0.0003 ; $n = 3$) than in controls. **K, L**, Nissl stainings of sagittal sections of the cerebral cortex at three anatomic levels at P21 reveals no obvious difference in thickness between wild types and mutants. **M, N**, Staining with antibodies to Tbr1 (green) to reveal layer II/III and VI neurons. **O, P**, Staining with antibodies to Cux1 (red) to reveal layer II–IV neurons. **Q**, Quantification of the thickness of the cortex at three anatomical levels reveals no difference between wild type and mutants. **R, S**, The density of Cux1- and Tbr1-positive neurons at P8 show no difference between wild types and mutants. All values are mean $\pm$ SD. Asterisks indicate significant differences. Scale bars, 100 μ m.

revealed a decline that was delayed relative to the loss of Pax6-positive cells and therefore likely a secondary consequence of RGC loss (Fig. 2*J–R*). Accordingly, whereas a significant loss of Pax6-positive cells was observed by E13, a reduction in the number of Tbr2-positive cells was evident by E16 (Fig. 2*I, R*).

Normal cell proliferation but enhanced apoptosis in *Itgb1-CNSko* mice

The decline in the number of neural precursors could be a direct consequence of changes in cell proliferation or cell death. We therefore used BrdU pulse-labeling (to label cells in

Figure 2. Decreased numbers of Pax6-positive RGC cells and Tbr2-positive transient amplifying cells. **A–H**, Coronal sections from E11–E18 brains stained with antibodies to Pax6 (red) to detect RGCs; nuclei were stained with DAPI (blue). **I**, Quantification of Pax6-positive RGCs shows a significant loss at E13, E16, and E18 in mutant mice ($p = 0.0078$, 0.0253 , and 0.0003). **J–Q**, Coronal sections from E11–E18 brains stained with antibodies to Tbr2 (green) to detect transient amplifying cells. **R**, Quantification of Tbr2-positive cells shows a significant loss at E16 and E18 in mutant mice. All values are mean $\pm$ SD. Asterisks indicate significant differences [$p = 0.0206$ (E16); 0.0008 (E18); $n = 2$]. Scale bars, $100 \mu\text{m}$.

S-phase) combined with Ki67 staining (to label proliferating cells throughout all stages of the cell cycle) to determine the labeling index (the proportion of BrdU-labeled cells among Ki67-positive cells) and the fraction of cells leaving the mitotic cycle (the quitting fraction, which is the proportion of Ki67 negative cells among BrdU-labeled cells). Timed pregnant females at E13 were injected with BrdU, embryos were collected 12 h later and sections were stained for BrdU and Ki67 (supplemental Fig. 2*A,B*, available at www.jneurosci.org as supplemental material). No differences were observed in labeling index and quitting fraction between wild-type and mutant animals (supplemental Fig. 2*I,J*, available at www.jneurosci.org as supplemental material). To distinguish proliferating RGCs from basal progenitors, we also stained histological sections with antibodies to Pax6 and Ki67 (supplemental Fig. 2*C–H*, available at www.jneurosci.org as supplemental material) and determined the number of double positive cells (supplemental Fig. 2*K*, available at www.jneurosci.org as supplemental material). There was no difference in the percentage of double positive cells between wild-type and mutant animals. Analysis of interkinetic nuclear migration also revealed no major defect in the mutant animals (supplemental Fig. 2*L–T*, available at www.jneurosci.org as supplemental material).

To determine whether the defects in cortical size might be caused by cell death, we stained histological sections by the TUNEL method. Whereas few apoptotic cells were present in the ventricular zone of wild-type mice, their number was drastically enhanced in the mutants. An increase in apoptosis was observed at E11 and E13 (Fig. 3*A–C,F,G,J,L*), but not at E15 (Fig. 3*H,I*) and P5 (supplemental Fig. 3, available at www.jneurosci.org as supplemental material). Staining with antibodies to activated caspase 3 confirmed that cell death was increased at E11, and costaining with antibodies to caspase 3 and Pax6 confirmed that the dying cells were RGCs (Fig. 3*D,E,D',E'*). We conclude that the loss of RGCs in *Itgb1-CNSko* mice is a consequence of enhanced apoptosis that occurs before but not after E15.

Cell death follows detachment of RGC processes from the meninges

We hypothesized that RGC death might be caused by loss of contact between RGC processes and meningeal BM components. To test this model, we followed the fate of individual

Figure 3. Increased apoptosis of RGC. *A–C*, Coronal sections from the E11 telencephalon stained with TUNEL (red) to reveal apoptotic cells. Nuclei stained with DAPI (blue). The cortical surface is outlined with a dashed line. *D, E*, Coronal sections from the E11 telencephalon stained with antibodies to activated caspase-3 (red) to reveal apoptotic cells and Pax6 (green) to reveal RGCs. *D', E'*, As *D* and *E* but showing the green channel only. Arrows in *E'* point to cells that are positive for activated caspase-3 and Pax6. *F, G*, Coronal sections from the E13 telencephalon stained with TUNEL (red) to reveal apoptotic cells. Nuclei are stained with DAPI (blue). The cortical surface is outlined with a dashed line. *H, I*, Coronal sections from E15 brains. Staining of apoptotic cells is performed using antibodies against activated caspase-3 (red), nuclei are stained with DAPI (blue). The cortical surface is outlined with a dashed line. *J–L*, Quantification of TUNEL and caspase 3 positive cells in the ventricular zone shows a significant increase in *Itgb1-CNSko* brains at E11 and E13. All values are mean $\pm$ SD. Asterisks indicate significant differences (*J*, $p = 0.01512$; *K*, $p = 0.02651$; *L*, $p = 0.03912$). Scale bars, 100 μ m.

RGCs over time. We generated a plasmid vector for the expression of a bicistronic CRE-IRES-EGFP mRNA. Electroporation of this construct into the brain of embryonic *Itgb1^{lox/lox}* mice is expected to lead to the inactivation of the *Itgb1* gene in EGFP-positive cells, permitting to observe their morphology and fate by time-lapse video microscopy. To test our expression vector, we electroporated brains from E11 and E13 wild-

Figure 4. Detachment and disintegration of RGC processes. **A, B**, Brains from wild-type and *Itgb1*^{fl/fl} mice at E11 were electroporated with a CRE-IRES-EGFP expression vector and analyzed 24 h later for EGFP fluorescence. Arrowheads point to detached RGC processes that were only visible in slices from *Itgb1*^{fl/fl} mice. **C, D**, Still images from time-lapse recordings at the indicated time after electroporation with CRE-IRES-EGFP. The cortex is outlined by dotted lines. In slices from wild-type mice, RGC cell bodies that were brightly positive for EGFP (red arrowheads) were observed throughout the VZ, but unlike in the confocal images of fixed sections (**A, B**), RGC processes were too dimly labeled and not visible. During the time course of the recording, RGC cell bodies in wild-type mice remained brightly fluorescent, but some migrated for small distances. In slices from *Itgb1*^{fl/fl} mice, brightly labeled cell bodies disappeared over time. **E, F**, Brains from wild-type and *Itgb1*^{fl/fl} mice at E13 were electroporated with a CRE-IRES-EGFP expression vector and analyzed 24 h later for EGFP fluorescence. Arrowheads point to detached RGC processes that were only visible in slices from *Itgb1*^{fl/fl} mice. **G, H**, Still images from time-lapse recordings shown in supplemental Movies 1 and 2, available at www.jneurosci.org as supplemental material. Times after electroporation with CRE-IRES-EGFP are indicated. In slices from wild-type mice, RGC processes were anchored at the meninges and remained stable during the entire observation period. In slices from *Itgb1*^{fl/fl} mice, RGC processes detach and disintegrated. Three processes are highlighted with arrowheads. Scale bars: **A, B**, 100 μ m; **C, D**, 50 μ m.

type mice and *Itgb1*^{fl/fl} mice and analyzed EGFP-positive RGCs after 24 h in fixed sections. Whereas RGC processes in sections from wild-type mice spanned the cortical wall and formed endfeet at the meningeal BM (Fig. 4*A, E*), glial processes in slices from *Itgb1*^{fl/fl} mice were stunted and lacked endfeet (Fig. 4*B, F*), indicating that our expression vector effectively inactivated β 1 integrin function in RGCs.

Next, we electroporated brains from E11 animals and followed the fate of individual RGCs by time-lapse video microscopy in cortical slices. Because the ventricular zone at E11/E12 spans most of the developing cortical wall, the bright EGFP signal from the cell bodies did not allow us to trace the weakly fluorescent radial processes of individual RGCs during live cell imaging (Fig. 4*C, D*). However, we observed that whereas the number of EGFP-positive cell bodies remained largely unchanged in slices from wild-type mice, their numbers drastically decreased over time in slices from *Itgb1*^{fl/fl} mice (Fig. 4*C, D*).

To achieve higher resolution in our imaging studies, we

next performed time-lapse experiments at E13/E14, a time point when cell death was still prominent in the cortex of *Itgb1*-CNSko mice (Fig. 3*F, G, L*). By E13/E14, the cortical plate has substantially expanded in thickness, allowing the live imaging analysis of RGC processes within the cortical wall without interference of EGFP signals from cell bodies in the ventricular zone (Fig. 4*G, H*). In slices from wild-type mice, the vast majority of EGFP-positive RGC processes were maintained throughout the entire observation period (Figs. 4*G*, 5*A*; supplemental Movie S1, available at www.jneurosci.org as supplemental material). A strikingly different result was obtained with slices from *Itgb1*^{fl/fl} mice. RGC processes showed bright EGFP fluorescence at the onset of the observation period, but they detached from the meninges and subsequently disintegrated. Three representative RGC processes are highlighted by arrows in Figure 4*H*, and also shown in supplemental Movie S2, available at www.jneurosci.org as supplemental material. The RGC processes of each of the three cells detached from the meninges within less than a day and formed

a bulbous ending that terminated within the cortical wall. After their initial detachment, the RGC processes maintained their length and shape and did not retract any further. Instead, the EGFP fluorescence signal fragmented into individual bright puncta followed by complete loss of fluorescence, indicative of disintegration of the RGC processes. A time-lapse series demonstrating detachment and subsequent disintegration of a single RGC process is shown in Figure 5*B*. In each time-lapse series, we sampled images in a stack covering 4 μm above and below the brightest fluorescence signal, ensuring that the fibers did not simply drift out of focus.

We next analyzed not only the processes of RGCs but also their cell bodies. To achieve optimal resolution, we focused on areas of the cortex where only few cells had been transfected, thereby allowing us to image simultaneously the processes and the far brighter cell bodies of individual cells (Fig. 5*C*; supplemental Movie S3, available at www.jneurosci.org as supplemental material). Whereas RGC processes and cell bodies in slices from electroporated wild-type mice remained stable (Fig. 5*A*), processes of RGCs in slices from electroporated *Itgb1^{flox/flox}* mice started to disintegrate within 28.9 ± 10.8 h after electroporation ($n = 76$) (Fig. 5*B*). Fragmentation of the cell body was observed with a delay [38.4 ± 14.5 h after electroporation ($n = 29$)] (Fig. 5*C*). Based on our real-time imaging data and the study of cell death using TUNEL and caspase 3 stainings, we conclude that RGCs that have lost contact with the meningeal BM undergo apoptosis, in which the cell processes disintegrate followed by fragmentation of the cell bodies.

Removal of the meninges and their associated blood vessels causes apoptosis

Loss of $\beta 1$ integrins may have affected RGCs in several ways that could ulti-

←

Figure 5. Disintegration of cell bodies follows disintegration of RGC processes. The still images are derived from supplemental Movies 3–5, available at www.jneurosci.org as supplemental material. **A**, Time-lapse images captured after CRE-IRES-EGFP electroporation into a brain of a wild-type mouse showing stable RGC processes throughout the observation period. **B**, **C**, Time-lapse images after CRE-IRES-EGFP electroporation into a brain of a *Itgb1^{flox/flox}* mouse. The arrow in **B** points to the tip of an RGC process that detaches from the meninges, retracts into the cortical wall, and subsequently disappears. The arrow in **C** points to the cell body of an RGC that disintegrates after the disappearance of the RGC process. Scale bars, 20 μm .

Figure 6. Surgical removal of the meninges and associated blood vessels triggers cell death. **A, B**, Brain slice from E13 animals cultured for 36 h with (ctrl, control) (**A**) or without (**B**) the meninges. BM were labeled with antibodies to laminin (red), the CR cells with antibodies to reelin (green), and the nuclei of cells with DAPI (blue). Note that after removal of the meninges and associated blood vessels (**B**), laminin expression is no longer detected and the CR cell layer is less well organized. **C, D** TUNEL staining (red) reveals that removal of the meninges and associated blood vessels at E13 leads to cell death in the ventricular zone. **E, F**, TUNEL staining (red) reveals no increase in cell death after removal of meninges and associated blood vessels at E15. Scale bars, 50 μ m. v, Ventricle.

mately lead to their death. To obtain additional evidence that contact of RGC processes with the meninges is required to sustain the survival of RGCs, we electroporated E13.5 wild-type embryos with an EGFP expression vector, prepared slice cultures, and used a surgical knife to make an incision just below the meninges to transect RGC processes. Analysis of EGFP fluorescence 16 h later demonstrated that the truncated RGC fibers retracted into the cortical wall (supplemental Fig. 4A,B, available at www.jneurosci.org as supplemental material). When we stained the sections subsequently with the TUNEL method, we observed cell death in the ventricular zone (supplemental Fig. 4C,D,C',D', available at www.jneurosci.org as supplemental material). Cell death was confined to the ventricular zone below the cortical region where RGC processes had been transected, and was not observed in adjacent regions that contained intact RGC processes. Note that areas where cell death occurred contained reduced number of EGFP-positive cells, because the EGFP signal drastically diminished while cells were undergoing apoptosis (supplemental Fig. 4, available at www.jneurosci.org as supplemental material).

As surgical transections may have caused injury to RGC that triggered their death, we also cultured slices from E13 animals after surgical removal of the meninges and their associated blood vessels, avoiding other damage to the slices. Staining with 4',6'-diamidino-2-phenylindole dihydrochloride (DAPI) and antibodies to laminin confirmed that BM components that are associated with the meninges and their blood vessels had been removed (Fig. 6A,B). Staining with antibodies to reelin revealed that CR cells were still present, although the CR cell layer was less well maintained compared with untreated slices, and some CR cells invaded the cortical wall (Fig. 6A,B). However, TUNEL staining revealed massive cell death that was largely confined to the ventricular zone (Fig. 6C,D). As we observed elevated cell death in *Itgb1-CNSko* mice only during early stages of corticogenesis, we also removed the meninges and their associated blood vessels from slices prepared from E15 mice. Importantly, the surgical procedure no longer caused apoptosis (Fig. 6E,F). Together, our findings suggest that interactions of RGCs processes with the meninges are critical for RGC survival during early stages of cortical development.

Combined inactivation of laminin $\alpha 2$ and $\alpha 4$ results in increased apoptosis and reduced cortical size

Previous studies suggest $\beta 1$ -integrins that bind laminin might be involved in attachment of RGC processes at the meningeal BM (Georges-Labouesse et al., 1998; De Arcangelis et al., 1999). We therefore analyzed the cerebral cortex in mice with mutations in laminin $\alpha 2$ and $\alpha 4$ subunit genes. These mice were chosen because both α subunits are expressed in the meninges and their blood vessels (Blaess et al., 2004). In contrast to mice with mutations in other laminin subunit genes such as $\alpha 1$ and $\beta 2$ (Miner and Yurchenco, 2004), the mutant mice are not embryonically lethal (Yang et al., 2005), allowing an analysis of cortical size. The cortex of mice with mutations in either the laminin $\alpha 2$ or $\alpha 4$ subunit genes did not show any obvious defect in size and morphology, but cortical size was significantly affected in $\alpha 2/\alpha 4$ double mutants (Fig. 7A–D). Similar to *Itgb1-CNSko* mice, the length of the cerebral cortex was reduced by $14.8 \pm 0.9\%$, whereas cortical thickness appeared unaffected (Fig. 7E,F). TUNEL stainings of sagittal sections obtained from E11 $\alpha 2/\alpha 4$ double mutants also revealed a drastic enhancement in apoptosis throughout the ventricular neuroepithelium (Fig. 7G,H,I). Analysis of the morphology of RGC processes by staining with an antibody to RC2 confirmed that their processes were similarly detached as previously reported for *Itgb1-CNSko* mice (Fig. 7J,J',K,K'). The laminin $\alpha 2$ subunit is a component of laminin-2, -4, and -12 isoforms,

Figure 7. Reduction in cortical size, increased cell death, and detachment of RGC processes in mice lacking laminin $\alpha 2$ and $\alpha 4$. **A–D**, Nissl-stained sagittal sections of P21 brains from wild-type and laminin mutants. Note that the brains of mice lacking both laminin $\alpha 2$ and $\alpha 4$ (LNa2/a4-KO) are significantly reduced in size. **E**, Quantification of the length of the cerebral cortex at P21 shows a significantly shorter cortex in LNa2/a4-KO mice (wt 8.16 ± 0.23 , LNa2-KO 7.81 ± 0.14 ; LNa2/a4-KO 7.01 ± 0.07 ; $n = 3$; $p = 0.0005$). **F**, Quantification of the thickness of the cerebral cortex at P21 shows no significant difference between wild-type and mutant mice. **G–I**, Coronal sections of the developing cortex of E11 wild-type and LNa2/a4-KO mice were stained with TUNEL (red). Nuclei were stained with DAPI (blue). Quantification of TUNEL stained apoptotic cells shows a significant increase of apoptosis in LNa2/a4-KO brains (**G**; wt 1.38 ± 0.53 ; LNa2/a4-KO 6.63 ± 0.53 ; $n = 2$; $p = 0.005$). All values are mean $\pm$ SD. Asterisks indicate significant differences. **J, K**, RGC processes in wild-type and LNa2/a4-KO mice were stained with antibodies to RC2, and the meningeal BM was visualized with an antibody that detects laminin 1 and 2. Note that the RGC processes (arrowheads) in LNa2/a4-KO mice are detached from the meninges. Scale bars, 100 μ m.

whereas the $\alpha 4$ subunit is a component of laminin-8 and -9 isoforms, suggesting that several of these isoforms have redundant functions in regulating cell survival.

Discussion

Here, we show that radial RGC processes have a previously unappreciated function in the regulation of RGC survival that ultimately affects the growth of the cerebral cortex. In *Itgb1-CNSko* mice, anchorage of RGCs at the meningeal BM is disrupted, leading to retraction of RGC processes into the cortical wall and the subsequent apoptosis of RGCs. Apoptosis is also induced in cortical slices from wild-type mice by removal of the meninges and their associated blood vessels. Finally, detachment of RGC processes, enhanced apoptosis, and a reduction in the size of the

cerebral cortex are observed in mice deficient in both laminin $\alpha 2$ and $\alpha 4$. Interestingly, $\beta 1$ -integrin-mediated contact with the meningeal BM is essential for RGC survival during early stages of cortical development, when symmetric cell divisions increase the size of the RGC pool. As a consequence, the decrease in the total number of RGCs leads to a reduction in cortical surface area in the lateral and longitudinal dimension. In contrast, maintenance and differentiation of RGCs during subsequent stages of cortical development, when RGC mainly generate neurons and astrocytes, is not affected. Therefore, cortical cell layers of normal thickness containing appropriate numbers of differentiated neurons develop normally in *Itgb1-CNSko* mice.

The generation, differentiation, and survival of RGCs de-

pend on local interactions in the ventricular neuroepithelium that are mediated by signaling molecules such as notch and Eph/Ephrin (Gaiano et al., 2000; Depaepe et al., 2005; Yoon et al., 2008). However, the function of RGC processes in the control of cell proliferation, differentiation, and survival has remained unclear. Whereas chemical ablation or genetic disruption of interactions between RGCs and the meninges affect cortical lamination, a direct role of the meninges in the control of RGC proliferation or survival has not been demonstrated (Pehlemann et al., 1985; Sievers et al., 1985, 1986; von Knebel Doeberitz et al., 1986; Hartmann et al., 1992; Georges-Labouesse et al., 1998; Miner et al., 1998; Costell et al., 1999; De Arcangelis et al., 1999; Supèr et al., 2000; Graus-Porta et al., 2001; Halfter et al., 2002; Michele et al., 2002; Moore et al., 2002; Beggs et al., 2003; Poschl et al., 2004; Niewmierzycka et al., 2005). In fact, one previous study has shown that mutations that disrupt interactions between RGC processes and the meningeal BM do not affect proliferation and differentiation of RGC (Haubst et al., 2006). However, the analysis focused on the behavior of RGCs after E14, and the findings do not explain the reduction in brain size that are associated with mutations in $\beta 1$ integrins (this study), perlecan, and laminin $\gamma 1$ (Costell et al., 1999; Halfter et al., 2002; Girós et al., 2007). In addition, earlier studies relied on mice that carried mutations in the genes for perlecan and laminin $\gamma 1$ in all tissues. In contrast, we have disrupted interactions between RGCs and the meninges by conditional knock-out of $\beta 1$ integrins in RGCs, and we have analyzed cortical development starting at E11. In agreement with earlier findings (Haubst et al., 2006), we show that after E15, contact of radial RGC processes with the meninges does not affect RGC proliferation, survival, and differentiation. Instead, $\beta 1$ integrins are critical for RGC survival during early stages of cortical development when symmetric cell divisions amplify the RGC pool. Accordingly, effects on cell survival are prominent at E11, when symmetric cell divisions prevail, prominent but less pronounced at E13, when a large number of RGCs still undergo symmetric cell divisions, and finally undetectable at E15, when RGCs have mainly shifted to the generation of neurons. Cell cycle progression of RGCs is not affected in the mutant mice, suggesting that defects in the size of the cerebral cortex in *Itgb1-CNSko* mice are largely caused by apoptosis of RGC. Interestingly, previous studies have shown that Eph/Ephrin signaling is also required to sustain RGC survival between E11.5 and E12.5 but not at subsequent ages (Depaepe et al., 2005). These findings suggest that the signals that control RGC survival change during early and late stages of cortical development.

Studies with cultured cells and genetically modified mice have demonstrated that loss of integrin-mediated attachment frequently leads to activation of signaling pathways that cause cell death, a process that has been termed anoikis (Frisch and Ruoslahti, 1997). Whereas *in vitro* studies with neurospheres have shown that integrin-dependent signals control the survival and proliferation of neural stem cells (Campos et al., 2004; Leone et al., 2005), it is currently unclear whether detachment of RGC processes leads to anoikis. Based on several observations, we consider it more likely that cell death in $\beta 1$ -deficient RGCs is caused by a different mechanism. First, anoikis is initiated within minutes after detachment (Frisch and Ruoslahti, 1997). In contrast, we observed death of RGCs only many hours after detachment of RGC processes. Second, although $\beta 1$ integrins were inactivated in the vast majority of

RGCs of *Itgb1-CNSko* mice, only a fraction of RGCs died, suggesting that not all RGCs are equally vulnerable to the loss of $\beta 1$ integrins. In *Itgb1-CNSko* mice, RGC processes retract to varying degree into the cortical wall, where some remained in close proximity to the meninges (Graus-Porta et al., 2001). We observed in time-lapse recordings apoptosis of those RGCs that retracted their processes for a considerable distance into the cortical wall. Although further studies are necessary, we favor the hypothesis that RGC processes are required to receive a trophic factor, and that $\beta 1$ integrins function to keep RGC endfeet in close apposition to cells presenting the signal. This scenario provides an interesting parallel to the control of cell survival in the peripheral nervous system, where target-derived neurotrophic factors that are retrogradely transported along the axon rescue the cell body from apoptotic cell death (Reichardt, 2006; Cosker et al., 2008). In preliminary studies, we have not been able to rescue RGCs from death by application of meningeal cell-conditioned medium, but further studies are required to test this model more thoroughly.

Time-lapse video recordings have provided evidence that during proliferation only one of the two daughters of a RGC inherits the radial process, whereas the second daughter loses contact with the meninges, followed by regrowth of a radial process (Miyata et al., 2001; Noctor et al., 2001, 2002). Based on the findings presented here, one might anticipate that the daughter cell without a radial process might undergo apoptosis. However, apoptosis is initiated only several hours after detachment, suggesting that trophic signals are maintained for considerable time in RGCs, providing sufficient time for process regrowth. Survival signals might provide a safeguard mechanism to ensure that only those RGCs that established contact with the meninges are maintained. Cells with aberrant processes would be eliminated, preventing the misrouting of migrating neurons to inappropriate areas. It should also be noted that different classes of RGC have been described previously (Pinto and Götz, 2007), raising the possibility that only some RGCs are affected by defects in the attachment of their processes.

Our findings extend earlier studies that have implicated the cortical marginal zone as a signaling center for cortical development. As outlined in the introduction, CR cell have a critical function in cortical development, affecting the differentiation of RGCs and secreting reelin, which is required for the development of the normal laminar structure of the cerebral cortex. We show here that detachment of RGC processes from the meningeal BM leads to apoptosis of RGCs. Cell death is also induced when the meninges and their associated blood vessels are surgically removed. In addition, studies with cells in culture suggest that the meninges can regulate cell proliferation and neuronal differentiation (Barakat et al., 1981; Gensburger et al., 1986). However, the cell types that provide signals for RGCs still need to be determined. The inner-most layer of the meninges, the pia, produces the BM covering the cortex and serves as the source for blood vessels in the superficial region of the cerebral cortex. The middle layer, the arachnoid, has important function in the resorption of CSF, whereas the outermost layer, the dura, is a collagenous structure that is tightly attached to the calvarium (McLone and Bondareff, 1975; Bauer et al., 1993; Sievers et al., 1994). The BMs at the cortical surface and surrounding endothelial cells contain laminin, where laminin $\alpha 2$ is prominent in the BM at the cortical surface and $\alpha 4$ in the endothelial BM (Blaess et al., 2004; Hallmann et al., 2005). Therefore, in *Itgb1-CNSko* mice and in

laminin $\alpha 2/\alpha 4$ double mutants, interactions of RGC processes with both BM assemblies are likely disrupted. Signaling molecules that affect RGCs could therefore be derived from several sources including meningeal fibroblasts, endothelial cells, the CSF, and the blood stream.

References

- Barakat I, Wittendorp-Rechenmann W, Rechenmann RV, Sensenbrenner M (1981) Influence of meningeal cells on the proliferation of neuroblasts in culture. *Dev Neurosci* 4:363–372.
- Bauer HC, Bauer H, Lametschwandner A, Amberger A, Ruiz P, Steiner M (1993) Neovascularization and the appearance of morphological characteristics of the blood-brain barrier in the embryonic mouse central nervous system. *Brain Res Dev Brain Res* 75:269–278.
- Beggs HE, Schahin-Reed D, Zang K, Goebbels S, Nave KA, Gorski J, Jones KR, Sretavan D, Reichardt LF (2003) FAK deficiency in cells contributing to the basal lamina results in cortical abnormalities resembling congenital muscular dystrophies. *Neuron* 40:501–514.
- Blaess S, Graus-Porta D, Belvindrah R, Radakovits R, Pons S, Littlewood-Evans A, Senften M, Guo H, Li Y, Miner JH, Reichardt LF, Müller U (2004) Beta1-integrins are critical for cerebellar granule cell precursor proliferation. *J Neurosci* 24:3402–3412.
- Campos LS, Leone DP, Relvas JB, Brakebusch C, Fässler R, Suter U, French-Constant C (2004) Beta1 integrins activate a MAPK signaling pathway in neural stem cells that contributes to their maintenance. *Development* 131:3433–3444.
- Cosker KE, Courchesne SL, Segal RA (2008) Action in the axon: generation and transport of signaling endosomes. *Curr Opin Neurobiol* 18:270–275.
- Costell M, Gustafsson E, Aszódi A, Mörgelin M, Bloch W, Hunziker E, Addicks K, Timpl R, Fässler R (1999) Perlecan maintains the integrity of cartilage and some basement membranes. *J Cell Biol* 147:1109–1122.
- De Arcangelis A, Mark M, Kreidberg J, Sorokin L, Georges-Labouesse E (1999) Synergistic activities of alpha3 and alpha6 integrins are required during apical ectodermal ridge formation and organogenesis in the mouse. *Development* 126:3957–3968.
- Depaepe V, Suarez-Gonzalez N, Dufour A, Passante L, Gorski JA, Jones KR, Ledent C, Vanderhaeghen P (2005) Ephrin signalling controls brain size by regulating apoptosis of neural progenitors. *Nature* 435:1244–1250.
- Fishell G, Kriegstein AR (2003) Neurons from radial glia: the consequences of asymmetric inheritance. *Curr Opin Neurobiol* 13:34–41.
- Frisch SM, Ruoslahti E (1997) Integrins and anoikis. *Curr Opin Cell Biol* 9:701–706.
- Gaiano N, Nye JS, Fishell G (2000) Radial glial identity is promoted by Notch1 signaling in the murine forebrain. *Neuron* 26:395–404.
- Gensburger C, Labourdette G, Sensenbrenner M (1986) Influence of meningeal cells on the proliferation and maturation of rat neuroblasts in culture. *Exp Brain Res* 63:321–330.
- Georges-Labouesse E, Mark M, Messaddeq N, Gansmüller A (1998) Essential role of alpha 6 integrins in cortical and retinal lamination. *Curr Biol* 8:983–986.
- Girós A, Morante J, Gil-Sanz C, Fairén A, Costell M (2007) Perlecan controls neurogenesis in the developing telencephalon. *BMC Dev Biol* 7:29.
- Götz M, Huttner WB (2005) The cell biology of neurogenesis. *Nat Rev* 6:777–788.
- Graus-Porta D, Blaess S, Senften M, Littlewood-Evans A, Damsky C, Huang Z, Orban P, Klein R, Schittny JC, Müller U (2001) Beta1-class integrins regulate the development of laminae and folia in the cerebral and cerebellar cortex. *Neuron* 31:367–379.
- Halfter W, Dong S, Yip YP, Willem M, Mayer U (2002) A critical function of the pial basement membrane in cortical histogenesis. *J Neurosci* 22:6029–6040.
- Hallmann R, Horn N, Selg M, Wendler O, Pausch F, Sorokin LM (2005) Expression and function of laminins in the embryonic and mature vasculature. *Physiol Rev* 85:979–1000.
- Hand R, Bortone D, Mattar P, Nguyen L, Heng JJ, Guerrier S, Boutt E, Peters E, Barnes AP, Parras C, Schuurmans C, Guillemot F, Polleux F (2005) Phosphorylation of Neurogenin2 specifies the migration properties and the dendritic morphology of pyramidal neurons in the neocortex. *Neuron* 48:45–62.
- Hartfuss E, Förster E, Bock HH, Hack MA, Leprince P, Luque JM, Herz J, Frotscher M, Götz M (2003) Reelin signaling directly affects radial glia morphology and biochemical maturation. *Development* 130:4597–4609.
- Hartmann D, Sievers J, Pehlemann FW, Berry M (1992) Destruction of meningeal cells over the medial cerebral hemisphere of newborn hamsters prevents the formation of the infrapyramidal blade of the dentate gyrus. *J Comp Neurol* 320:33–61.
- Haubst N, Georges-Labouesse E, De Arcangelis A, Mayer U, Götz M (2006) Basement membrane attachment is dispensable for radial glial cell fate and for proliferation, but affects positioning of neuronal subtypes. *Development* 133:3245–3254.
- Hunter KE, Hatten ME (1995) Radial glial cell transformation to astrocytes is bidirectional: regulation by a diffusible factor in embryonic forebrain. *Proc Natl Acad Sci U S A* 92:2061–2065.
- Leone DP, Relvas JB, Campos LS, Hemmi S, Brakebusch C, Fässler R, French-Constant C, Suter U (2005) Regulation of neural progenitor proliferation and survival by beta1 integrins. *J Cell Sci* 118:2589–2599.
- McLone DG, Bondareff W (1975) Developmental morphology of the subarachnoid space and contiguous structures in the mouse. *Am J Anat* 142:273–293.
- Michele DE, Barresi R, Kanagawa M, Saito F, Cohn RD, Satz JS, Dollar J, Nishino I, Kelley RI, Somer H, Straub V, Mathews KD, Moore SA, Campbell KP (2002) Post-translational disruption of dystroglycan-ligand interactions in congenital muscular dystrophies. *Nature* 418:417–422.
- Miner JH, Yurchenco PD (2004) Laminin functions in tissue morphogenesis. *Annu Rev Cell Dev Biol* 20:255–284.
- Miner JH, Cunningham J, Sanes JR (1998) Roles for laminin in embryogenesis: exencephaly, syndactyly, and placentalopathy in mice lacking the laminin alpha5 chain. *J Cell Biol* 143:1713–1723.
- Miyata T, Kawaguchi A, Okano H, Ogawa M (2001) Asymmetric inheritance of radial glial fibers by cortical neurons. *Neuron* 31:727–741.
- Moore SA, Saito F, Chen J, Michele DE, Henry MD, Messing A, Cohn RD, Ross-Barta SE, Westra S, Williamson RA, Hoshi T, Campbell KP (2002) Deletion of brain dystroglycan recapitulates aspects of congenital muscular dystrophy. *Nature* 418:422–425.
- Niewmierzyska A, Mills J, St-Arnaud R, Dedhar S, Reichardt LF (2005) Integrin-linked kinase deletion from mouse cortex results in cortical lamination defects resembling cobblestone lissencephaly. *J Neurosci* 25:7022–7031.
- Noctor SC, Flint AC, Weissman TA, Dammerman RS, Kriegstein AR (2001) Neurons derived from radial glial cells establish radial units in neocortex. *Nature* 409:714–720.
- Noctor SC, Flint AC, Weissman TA, Wong WS, Clinton BK, Kriegstein AR (2002) Dividing precursor cells of the embryonic cortical ventricular zone have morphological and molecular characteristics of radial glia. *J Neurosci* 22:3161–3173.
- Patton BL, Cunningham JM, Thyboll J, Kortessmaa J, Westerblad H, Edström L, Tryggvason K, Sanes JR (2001) Properly formed but improperly localized synaptic specializations in the absence of laminin alpha4. *Nat Neurosci* 4:597–604.
- Pehlemann FW, Sievers J, Berry M (1985) Meningeal cells are involved in foliation, lamination, and neurogenesis of the cerebellum: evidence from 6-hydroxydopamine-induced destruction of meningeal cells. *Dev Biol* 110:136–146.
- Pinto L, Götz M (2007) Radial glial cell heterogeneity—the source of diverse progeny in the CNS. *Prog Neurobiol* 83:2–23.
- Polleux F, Whitford KL, Dijkhuizen PA, Vitalis T, Ghosh A (2002) Control of cortical interneuron migration by neurotrophins and PI3-kinase signaling. *Development* 129:3147–3160.
- Pöschl E, Schlötzer-Schrehardt U, Brachvogel B, Saito K, Ninomiya Y, Mayer U (2004) Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development. *Development* 131:1619–1628.
- Reichardt LF (2006) Neurotrophin-regulated signalling pathways. *Philos Trans R Soc Lond B Biol Sci* 361:1545–1564.
- Sievers J, Pehlemann FW, Baumgarten HG, Berry M (1985) Selective destruction of meningeal cells by 6-hydroxydopamine: a tool to study

- meningeal-neuroepithelial interaction in brain development. *Dev Biol* 110:127–135.
- Sievers J, von Knebel Doeberitz C, Pehlemann FW, Berry M (1986) Meningeal cells influence cerebellar development over a critical period. *Anat Embryol (Berl)* 175:91–100.
- Sievers J, Pehlemann FW, Gude S, Berry M (1994) Meningeal cells organize the superficial glia limitans of the cerebellum and produce components of both the interstitial matrix and the basement membrane. *J Neurocytol* 23:135–149.
- Soriano E, Alvarado-Mallart RM, Dumesnil N, Del Río JA, Sotelo C (1997) Cajal-Retzius cells regulate the radial glia phenotype in the adult and developing cerebellum and alter granule cell migration. *Neuron* 18:563–577.
- Sunada Y, Bernier SM, Kozak CA, Yamada Y, Campbell KP (1994) Deficiency of merosin in dystrophic dy mice and genetic linkage of laminin M chain gene to dy locus. *J Biol Chem* 269:13729–13732.
- Supèr H, Del Río JA, Martínez A, Pérez-Sust P, Soriano E (2000) Disruption of neuronal migration and radial glia in the developing cerebral cortex following ablation of Cajal-Retzius cells. *Cereb Cortex* 10:602–613.
- von Knebel Doeberitz C, Sievers J, Sadler M, Pehlemann FW, Berry M, Halliwell P (1986) Destruction of meningeal cells over the newborn hamster cerebellum with 6-hydroxydopamine prevents foliation and lamination in the rostral cerebellum. *Neuroscience* 17:409–426.
- Xu H, Christmas P, Wu XR, Wewer UM, Engvall E (1994) Defective muscle basement membrane and lack of M-laminin in the dystrophic dy/dy mouse. *Proc Natl Acad Sci U S A* 91:5572–5576.
- Yang D, Bierman J, Tarumi YS, Zhong YP, Rangwala R, Proctor TM, Miyagoe-Suzuki Y, Takeda S, Miner JH, Sherman LS, Gold BG, Patton BL (2005) Coordinate control of axon defasciculation and myelination by laminin-2 and -8. *J Cell Biol* 168:655–666.
- Yoon KJ, Koo BK, Im SK, Jeong HW, Ghim J, Kwon MC, Moon JS, Miyata T, Kong YY (2008) Mind bomb 1-expressing intermediate progenitors generate notch signaling to maintain radial glial cells. *Neuron* 58:519–531.