GPR56 Regulates Pial Basement Membrane Integrity and Cortical Lamination

Shihong Li,1 Zhaohui Jin,* Samir Koirala,** Lihong Bu,*** Lei Xu,4 Richard O. Hynes,4 Christopher A. Walsh,3 Gabriel Corfas,2 and Xianhua Piao1

1Division of Newborn Medicine, Department of Medicine, 2Neurobiology Program, Children’s Hospital Boston, Harvard Medical School, 3Department of Neurology, Howard Hughes Medical Institute, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts 02115, and 4Howard Hughes Medical Institute, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

GPR56 is a member of the family of adhesion G-protein-coupled receptors that have a large extracellular region containing a GPS (G-protein proteolytic site) domain. Loss-of-function mutations in the GPR56 gene cause a specific human brain malformation called bilateral frontoparietal polymicrogyria (BFPP). BFPP is a radiological diagnosis and its histopathology remains unclear. This study demonstrates that loss of the mouse Gpr56 gene leads to neuronal ectopia in the cerebral cortex, a cobblestone-like cortical malformation. There are four crucial events in the development of cobblestone cortex, namely defective pial basement membrane (BM), abnormal anchorage of radial glial endfeet, mislocalized Cajal–Retzius cells, and neuronal overmigration. By detailed time course analysis, we reveal that the leading causal events are likely the breaches in the pial BM. We show further that GPR56 is present in abundance in radial glial endfeet. Furthermore, a putative ligand of GPR56 is localized in the marginal zone or overlying extracellular matrix. These observations provide compelling evidence that GPR56 functions in regulating pial BM integrity during cortical development.

Key words: GPR56; bilateral frontoparietal polymicrogyria; BFPP; basement membrane; cobblestone cortex; cortical lamination

Introduction

Using positional cloning, we previously demonstrated that mutations in the GPR56 gene cause a specific human brain malformation called bilateral frontoparietal polymicrogyria (BFPP) (Piao et al., 2004). BFPP is a recessively inherited genetic disorder of human cerebral cortical development, characterized by disorganized cortical lamination that is most severe in the frontal and parietal lobes (Piao et al., 2002; Chang et al., 2003). The histopathology of BFPP remains unclear. Our follow-up genotype–phenotype analysis in patients with BFPP and other similar polymicrogyria syndromes have demonstrated that all predicted pathogenic GPR56 sequence alterations are associated with polymicrogyria plus white matter defects and brainstem and cerebellar hypoplasia (Piao et al., 2005; Piao and Walsh, 2007). The findings of impaired myelination, small pons, and cerebellar hyperplasia are common in individuals with congenital muscular dystrophy syndromes; however, they present in very few other disorders (A. J. Barkovich, personal communication).

Cobblestone lissencephaly is defined as aberrant migration of cortical neurons out of the developing brain through breaches in the pial basement membrane (BM), forming neuronal ectopias on the surface of the brain (Olson and Walsh, 2002). Cobblestone lissencephaly is typically seen in three distinct human congenital muscular dystrophy syndromes, muscle–eye–brain disease (MEB), Fukuyama-type muscular dystrophy (FCMD), and Walker–Warburg syndrome (WWS) (Olson and Walsh, 2002). MEB, FCMD, and some WWS cases are caused by aberrant glycosylation of α-dystroglycan, a receptor for laminin (Kobayashi et al., 1998; Yoshida et al., 2001; Michele et al., 2002; Yamamoto et al., 2004). Mutant mice with deletions in some members of the integrin family, downstream associates of integrins, such as FAK (focal adhesion kinase) and Itk (integrin-linked kinase), or extracellular matrix constituents like the proteoglycan, perlecán, and the nidogen-binding site of laminin γ1, also show cortical migration defects with deficiencies in basal lamina integrity, features that resemble human cobblestone lissencephaly (Georges-Labouesse et al., 1998; Costell et al., 1999; De Arcangelis et al., 1999; Graus-Porta et al., 2001; Halfter et al., 2002; Beggs et al., 2003; Niewmierzycka et al., 2005; Haubst et al., 2006). To date, the majority of cobblestone cortex–causative genes identified encode proteins that are directly or indirectly involved in pial BM organization (Georges-Labouesse et al., 1998; Costell et al., 1999; De Arcangelis et al., 1999; Graus-Porta et al., 2001; Halfter et al., 2002; Beggs et al., 2003; Niewmierzycka et al., 2005; Haubst et al., 2006; Hu et al., 2007).
In this study, we show that loss of GPR56 leads to cortical lamination defects, with overmigration of neurons through the pial layer, demonstrating the histopathology of BFPP to be a cobblestone-like brain malformation. Furthermore, by analyzing detailed time courses to evaluate BM integrity and radial glial endfeet anchorage, we demonstrate that breaks in pial BM are likely the leading causal events, which are, in turn, associated with abnormal anchorage of radial glial endfeet. We further reveal that a putative ligand of GPR56 is present in the pial BM. Thus, our findings establish a role for GPR56 in regulating pial BM integrity and corticogenesis.

Materials and Methods

Mice. The Gpr56 knock-out mice were obtained from Genentech/Lexicon Genetics. The mutant mice were originally created in a 129/B6 background, but were rederived into the FvB strain and bred to BALB/c at one point. Therefore, the genetic background of the mutant mice is mixed: 129/B6FvB/BALB/c. The genotype of the mice was determined by PCR using the following primers: A (5’-CGAGAAGACTCCGGTTCTG), B (5’-AAAGTAGCTAAGATGCTCTCC), and Neo (5’-GCAGCGCAGTGGCCTCTTAC). The time point at 10:00 A.M. on the day of vaginal plug was defined as embryonic day 0.5 (E0.5). Fetal stage was calculated from the day when a vaginal plug was observed. All animals were treated according to the guidelines of the Animal Care and Use Committee of Children’s Hospital Boston.


Histology and immunohistochemistry. Embryos and postnatal mice were fixed or perfused with 4% paraformaldehyde. Brains were either cryoprotected by 30% sucrose, frozen over dry ice and sectioned on a cryostat, or embedded in paraffin and sectioned on a microtome. Sections were stained with 0.1% cresyl violet/0.5% acetic acid, or processed for immunostaining using standard procedures. Primary antibodies were visualized by appropriate fluorophore-conjugated secondary antibodies (Invitrogen; 1:400). Nuclei were stained with Hoechst 33342 (Invitrogen). All images were captured using a confocal LSM 510 NLO system (Carl Zeiss) or a Nikon 80i upright microscope. Representative photographs were obtained with the same exposure setting for control and mutant.

BrdU labeling and quantitative analysis. Pregnant mice (E12.5, E15.5, and E17.5) were injected intraperitoneally with BrdU (50 mg/g body weight, dissolved in PBS). Brains were removed at postnatal day 1 (P1) and prepared for cryostat sections. Sections for BrdU staining were pre-
treated for 15 min in 2N HCl at 40°C. Quantification of newborn cells and distribution within cortical layers were done by using the QuikChange II XL Site-Directed Mutagenesis kit (Stratagene) according to the protocols suggested by the manufacturer. The primers for GPR56del were as follows: forward, 5’-GCCGGCCGGTGGTTG-CACAGGCGAGT-3’, and reverse, 5’-GCCGGCCGGTGGTTG-CACAGGCGAGT-3’. Deletion mutagenesis was done by using the QuikChange II XL Site-Directed Mutagenesis kit (Stratagene) according to the protocols suggested by the manufacturer. The primers for GPR56del were as follows: forward, 5’-GCCGGCCGGTGGTTG-CACAGGCGAGT-3’, and reverse, 5’-GCCGGCCGGTGGTTG-CACAGGCGAGT-3’.

Figure 2. Abnormal cortical lamination in Gpr6−/− mice. A. Immunostaining for cortical layer-specific markers in P6 mouse brains revealed the disorganized lamination in Gpr6−/− mice. Ectopic cluster comprises neurons from both deep and superficial layers. B. In vivo migration assay using BrdU pulse labeling at E12.5 (a, b), E15.5 (c, d), and E17.5 (e, f) revealed that neuronal proliferation and migration were mostly unaffected in Gpr6−/− cortex, except in the ectopic regions. Both deep and upper layer neurons migrated into the marginal zone. Scale bars, 100 μm.

Table 2. Location of cortical ectopias

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. of animals analyzed</th>
<th>Presence of cortical ectopias</th>
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<tbody>
<tr>
<td>P0.5</td>
<td>10</td>
<td>Yes</td>
</tr>
<tr>
<td>P6</td>
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<td>Yes</td>
</tr>
<tr>
<td>P14</td>
<td>3</td>
<td>Yes</td>
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<tr>
<td>6 weeks</td>
<td>4</td>
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Among the 27 Gpr6-null mutant animals analyzed, cortical ectopias were detected only in the frontaloparietal cortex.

Results

GPR56 is expressed in neural progenitors in murine fetal forebrain

We previously showed that Gpr56 mRNA is detected in the ventricular zone of the mouse developing cortex (Piao et al., 2004). To better localize GPR56 protein in developing forebrain, we stained sections of embryonic mouse brains at E12.5, E14.5, and E16.5 with an affinity-purified rabbit anti-mouse GPR56 antibody. The specificity of the GPR56 antibody was confirmed on cell lines transfected with a GPR56 expression construct, as well as on Gpr6 knock-out mouse brain sections (supplemental Fig. 1A, available at www.jneurosci.org as supplemental material). GPR56 immunoreactivity was detected across the entire lateral ventricular wall at E12.5 and E14.5 (Fig. 1A; supplemental Fig. 1B, available at www.jneurosci.org as supplemental material). In the developing cortex at E16.5, GPR56 signals were found in the ventricular zone (VZ), intermediate zone (IZ), and marginal zone (MZ) (supplemental Fig. 1D, available at www.jneurosci.org as supplemental material). Double immunohistochemistry (IHC) with anti-GPR56 antibody and a marker for migrating neurons, Tuj1 revealed that GPR56 protein is mostly absent from the migrating neurons (supplemental Fig. 1C, E, available at www.jneurosci.org as supplemental material).

The expression pattern of GPR56 protein is similar to the distribution of radial glial cells. To confirm that radial glia express GPR56, we performed double-label IHC with GPR56 antibody and a radial glial marker, RC2, as well as combined in situ hybridization for Gpr6 and BLBP IHC on mouse developing cortex at various embryonic stages. BLBP, a member of the CRAB (cell-
lar retinoic acid-binding protein)/FABP (fatty acid-binding protein) gene family, is expressed in radial glial cells (Feng et al., 1994). GPR56 protein was predominantly colocalized with radial glial cell markers (Fig. 1B; supplemental Fig. 2, available at www.jneurosci.org as supplemental material). This observation is consistent with the expression data generated from Gpr56 BAC transgenic mice (http://www.gensat.org/login.jsp). Together, our data indicate that GPR56 is expressed in radial glia as well as other neural progenitor cells.

Loss of GPR56 leads to regional lamination defects
To study the pathogenesis of BFPP, we obtained Gpr56 knockout mice from Genentech and Lexicon. The genetic background of the mutant mice is 129/Bl6/FVB/BALB/c. The targeting scheme is shown in supplemental Figure 3A (available at www.jneurosci.org as supplemental material). Briefly, Gpr56 exons 2 and 3 are replaced by IRES-lacZ/MCI-neo, which results in the deletion of the starting ATG and a frameshift. The homozygous Gpr56 mutant mice were viable, fertile, and recovered at Mendelian ratios (supplemental Table 1, available at www.jneurosci.org as supplemental material).

GPR56 is cleaved at the GPS (G-protein proteolytic site) domain into an N- and a C-terminal fragments, GPR56N and GPR56C (supplemental Fig. 3B, available at www.jneurosci.org as supplemental material) (Xu et al., 2006; Jin et al., 2007). Western blot analysis with an antibody against the C terminus of GPR56C failed to detect any signal in Gpr56−/− mouse brain, in contrast with wild-type and heterozygous brains, confirming that the targeting strategy results in a true null allele (supplemental Fig. 3C, available at www.jneurosci.org as supplemental material).

Although the overall brain sizes were comparable in mutant and control animals, homozygous mutants revealed frequent occurrence of neuronal ectopias manifested by overmigration of neurons through the marginal zone (Fig. 1D,F). The hippocampus was mostly unaffected in the mutant mice (Fig. 1F). Only homozygous mutant embryos were phenotypically affected, whereas heterozygotes appeared normal and were used as controls in most of the experiments. The phenotype showed full penetrance in a total of 81 homozygous animals from E12.8 to adulthood, whereas there was not a single ectopia observed in a total of 7 wild-type and 62 heterozygous mice (Table 1). Cortical

Figure 3. Defective BM leads to neuronal ectopias. A–D, Double-label IHC of Tuj1 and laminin on E12.5 coronal sections revealing intact BM in both control and mutant animals. E–P, Double-label IHC of Tuj1 and various BM constituents on E13.5 coronal sections revealed a continuous thin lining of the BM on the cortical surface in the heterozygous brains (E, I, M) and postmitotic neurons positioned underneath the BM (F, J, N). In contrast, regionally ruptured BM was detected in the homozygous brains (G, K, O, arrows). Tuj1-positive neurons migrated through the defective BM (H, L, P, arrows). Scale bars, 100 μm.
lamination was disorganized in the areas beneath the ectopic outgrowths. In all of the postnatal Gpr56 null mutant animals analyzed, cortical ectopias were only detected in the frontoparietal cortex (Table 2).

The ectopic neurons comprise deeper and upper layer neurons
To determine the cellular composition of the ectopias, we performed immunostaining with various layer-specific markers, Cux1 for layers II–IV, Tbr1 for layers II–IV and VI, CTIP2 for layer V, and Darpp32 for layer VI (Nieto et al., 2004; Molyneaux et al., 2005). Neurons positive for Cux1, Tbr1, CTIP2, or Darpp32 were detected in the ectopias, suggesting that the ectopic cells in the Gpr56+/− cortex were neurons from both deep and superficial cortical layers (Fig. 2A). In addition, calbindin-positive interneurons were also detected in the ectopias (supple-

Figure 4. Regional breaks of the pial BM in the absence of GPR56. A, B, Double IHC of laminin (red) and Tuj1 (green) at embryonic stage E12.8 revealing the continuous pial BM and the migrating neurons underneath in a heterozygous brain (A), whereas a linear break of the pial BM and ectopic neurons were detected in a Gpr56 mutant (B, arrow). C–E, Electron-microscopic views at E13.5 showing the continuous pial BM in heterozygous brain (C, red dashed line) and ectopic neurons migrating out through the broken pial BM in Gpr56 knock-out brain (D), D', Higher-magnification view of the boxed area in D showing radial glial endfeet extending out (arrows) through the broken pial BM (red dashed line). E, Higher-magnification view of the normal attachment of radial glial endfeet to the pial BM in heterozygous brain. Scale bars: (in B) A, B, 20 μm; (in D) C, D, 10 μm; (in D') D', E, 1 μm.

Defective pial basement membrance leads to neuronal overmigration
Defective pial BM, abnormal anchorage of radial glial endfeet, mislocalized Cajal–Retzius (CR) cells, and neuronal overmigration are the four crucial events that are involved in the development of cortical ectopias. To determine the sequence of these four events, we first studied the time course of ectopia formation from E12.5 to E16.5. By Nissl staining, the earliest time point when an ectopia could be detected was E13.5 (supplemental Fig. 6D, arrow; available at www.jneurosci.org as supplemental material). The laminar organization became progressively distorted from E13.5 to E16.5 (supplemental Fig. 6D, F, H, available at www.jneurosci.org as supplemental material).

The pial BM is a specialized extracellular matrix (ECM) that is formed adjacent to the radial glial endfeet. Meningeal fibroblasts contribute to the BM by secreting the majority of basal lamina constituents, including laminin, collage IV, nidogen, and the heparan sulfate proteoglycan (Sievers et al., 1994). Defective pial BM is thought to be the primary cause of cobblestone lissencephaly (Olson and Walsh, 2002). We therefore examined whether there were defects in the pial BM by probing for extracellular matrix constituents, laminin, collagen IV (Col IV), and nidogen on sections of E12.5 and E13.5 mutant and control brains. Interestingly, the BM appeared intact in the mutant brain at E12.5,
suggesting that the BM was assembled normally at early developmental stages (Fig. 3C).

At E13.5, however, we found areas of discontinuity in laminin, Col IV, and nidogen immunoreactivities at the pial surface of Gpr56−/− mouse brains (Fig. 3G, K, O). In contrast, laminin, Col IV, and nidogen immunoreactivities were distributed as a continuous thin lining on the surface of the cortical wall in control animals (Fig. 3E, I, M). Double immunolabeling with Tuj1 and ECM constituents demonstrated that neurons migrated through the defective BM (Fig. 3H, L, P).

It is intriguing that the pial BM was assembled during the early stage of cortical development, and subsequently broke at later stages in the absence of GPR56. One possible explanation is that the ECM polymers are fragile in the absence of GPR56 and cannot sustain the tension generated by the expanding cortical wall. To test this possibility, we performed more detailed time course and EM analyses. By IHC, we could detect a region with disrupted BM and overmigrating neurons as early as E12.8 (i.e., at 6:00 P.M. on day 12 after vaginal plugging) (Fig. 4B). Interestingly, it appeared that Tuj1-positive neurons migrated through a linear break of the pial BM (Fig. 4B, white arrowhead). By EM analysis, we detected a sheet of pial BM together with the attached radial glial endfeet folded over by a herd of overmigrating neurons (Fig. 4D′, red arrows).

**GPR56 mutation leads to abnormal position of radial glial endfeet**

Radial glial endfeet attach to the BM and form an adhesive lining at the pial surface (Rakic, 2003). Defects in pial BM could lead to abnormal anchorage of radial glial endfeet. To test this hypothesis, we investigated whether loss of GPR56 leads to disorganized/displaced radial glial endfeet by probing for RC2 and BLBP in E12.8 and E15.5 brains, respectively. In control brains, radial glial processes were aligned in parallel arrays throughout the cerebral wall, terminating in well-defined endfeet at the pial BM, which was visualized by laminin or collagen IV staining (Fig. 5A, C). In mutant developing cortex, however, the radial glial fibers were disorganized and extended beyond the pia mater into the neuronal ectopia in the areas where the BM was disrupted (Fig. 5B, D, F, arrowheads). Although we could not completely exclude the presence of retracted endfeet, the predominant abnormalities were protruded endfeet that extended through the defective BM.

**Loss of GPR56 leads to CR cell malpositioning**

CR cells secrete reelin, an ECM signaling molecule that is critical for proper positioning of postmitotic neurons during cortical development (Tissir and Goffinet, 2003). To determine whether CR cells are abnormally located in developing Gpr56−/− cortex, we compared the distribution of CR cells in brains of Gpr56+/− and Gpr56−/− animals using both reelin and calretinin to identify CR cells. In contrast with the relatively continuous single layer of CR cells at the marginal zone in E12.8 control animals (Fig. 6A, C), we found CR cells located beyond the pial surface on the defective BM and on the peak of the ectopic neuronal clusters (Fig. 6B, D, arrows). In E16.5 brains, in contrast with a single-cell layer of well-defined CR neurons at the outer surface of the cortex in control animals (Fig. 6E, G), we observed scattered CR cells within the ectopia (Fig. 6F, H). There was a CR cell gap at the stem of the ectopia where a stream of neurons were migrating through (Fig. 6F, H, between the arrows).

**Strong GPR56 expression in radial glial endfeet**

Having established a role for GPR56 in BM organization/maintenance, we next investigated whether GPR56 protein is present in the radial glial endfeet. Double immunostaining with antibodies against GPR56 and Col IV revealed GPR56 signals terminating...
at the Col IV-positive BM at E16.5 (Fig. 7C). We then performed double immunostaining of GPR56 and GLAST, as well as BLBP and GPR56 on adjacent sections because both antibodies are rabbit antisera. We observed strong GPR56 immunoreactivity in the radial glial endfeet (Fig. 7D–H). Thus, our data support the hypothesis that GPR56 protein is trafficked to radial glial endfeet for its potential function of organizing and/or maintaining the BM.

**GPR56 binds a putative ligand in pial BM**

GPR56 is an orphan G-protein-coupled receptor. To investigate the distribution of putative GPR56 ligands in the developing cortex, we engineered a fusion construct in which mouse IgG Fc tag was fused to the C terminus of GPR56N as illustrated in Figure 8A. The fusion construct was used to transfect HEK-293T cells. Secreted GPR56N-mFc protein in the conditioned medium was collected, concentrated, and verified by Western blot (Fig. 8B). The concentrated GPR56N-mFc protein was used to probe mouse E12.5 and E14.5 brains with the mFc protein as negative control. GPR56N-mFc fusion protein was found to bind the overlying pial surface of the cerebral cortex at all embryonic stages investigated (Fig. 8E, F). This binding pattern was highly specific and could be detected with GPR56N-mFc preparations as low as 40 µg/ml. In contrast, no signal was detected using unfused mFc preparations as high as 500 µg/ml (Fig. 8C, E). The GPR56N-mFc binding pattern likely indicates a local binding partner/GPR56 interaction at the marginal zone or overlying extracellular matrix that is necessary for regulating the pial BM integrity. To confirm the GPR56 binding partner was present in the pial basement membrane, we performed double immunostaining with GPR56N-mFc and laminin, a basement membrane constituent. As expected, the GPR56N-mFc staining was well colocalized with laminin staining in the pial basement membrane (supplemental Fig. 7A, available at www.jneurosci.org as supplemental material).

To further verify the specificity of above binding pattern, we created a mutant fusion protein by deleting amino acids 93–143, GPR56N_del-mFc (Fig. 8A). The mutant fusion construct was transfected into HEK-293T cells to produce GPR56N_del-mFc fusion protein. Western blot analysis revealed a glycosylated and secreted GPR56N_del-mFc protein that is ~5 kDa smaller than the wild-type GPR56N-mFc (Fig. 8B). There was no specific staining when GPR56N_del-mFc fusion proteins were used to probe mouse E12.5 and E14.5 brain sections, thus confirming that the binding pattern revealed by GPR56N-mFc is highly specific (Fig. 8D, G).

To investigate whether the organization of the putative ligand is disrupted in the absence of GPR56, we performed GPR56N-mFc staining in E14.5 Gpr56−/− and Gpr56+/− littermate brain...
sections. In contrast with the smooth, continuous GPR56 N−mFc binding in control animals, we found breakdown and fragmented GPR56 N−mFc binding in the regions of ectopias (supplemental Fig. 7 B, C, available at www.jneurosci.org as supplemental material). These results supported that GPR56 binds a putative ligand in the pial basement membrane and the organization of this putative ligand is disrupted within the ectopic regions of Gpr56−/− brains.

Discussion
BFPP is a radiological diagnosis. Its pathology is unclear and extremely difficult to study because of the unavailability of brain specimens from affected individuals. Genotype–phenotype analysis in patients with BFPP and other similar polymicrogyria syndromes, however, indicated that BFPP brains have features of cobblestone-like brain malformations (i.e., polymicrogyria, cerebellar hypoplasia, and white matter defect) (Piao et al., 2005; Piao and Walsh, 2007). Here, we demonstrate in a Gpr56 knockout mouse model that the histopathology of BFPP is indeed a cobblestone-like cortical malformation.

The role of GPR56 in pial BM organization
The pial BM is a specialized ECM that is formed adjacent to the radial glial endfeet. Meningeal fibroblasts contribute to the BM by secreting and organizing the majority of basal lamina constituents (Sievers et al., 1994). The sites of ECM protein synthesis and BM assembly are spatially distinct, suggesting cell-directed mechanisms that can specify the sites of ECM assembly. Indeed, emerging evidence indicates that receptors on the cell surface, such as integrins and dystroglycan, can orchestrate the assembly of the ECM (Schwarzbauer, 1999).

In this study, we establish the role of GPR56 in regulating the pial BM integrity by demonstrating that (1) GPR56 is present in abundance in radial glial endfeet (Fig. 7C,F,G); (2) GPR56 binds a putative ligand in the ECM (Fig. 8E,H); and (3) loss of GPR56 in mice result in defective pial BM and neuronal ectopia, a cobblestone-like cortex. However, it remains to be determined whether GPR56 exerts its function by regulating ECM assembly or remodeling. The presence of intact BM in Gpr56 mutant mice during early embryonic stages suggests that the initial assembly of the pial BM occurs in the absence of GPR56 (Fig. 3C). Regional pial BM breakdown starts at E12.8, concurrent with the beginning of corticogenesis and cortical wall expansion. Therefore, it is possible that the ECM polymers are fragile in the absence of GPR56 and cannot sustain the tension generated by the expanding cortical wall. Indeed, we detected linear breaks in Gpr56−/− cortex at E12.8 (Fig. 4B) and folded pial BM together with the attached radial glial endfeet at E13.5 (Fig. 4D). Alternatively, loss of GPR56 could cause disturbed homeostasis in ECM remodeling. ECM remodeling is critical for many developmental processes as well as tumor metastasis. The establishment of homeostasis in ECM synthesis and proteolytic degradation is the key in ECM maintenance (Larsen et al., 2006).

GPR56 regulates the proper anchorage of radial glial endfeet
In the developing cerebral cortex, radial glial cells serve dual functions as progenitor cells that give rise to neurons as well as “guiding wires” that provide the organizing framework for corticogenesis (Malatesta et al., 2003; Rakic, 2003). Radial glial cells have their somata in the ventricular zone and extend their long radial processes through the entire developing cortex to terminate at the pial BM. The pial BM is formed at the interface between radial glial endfeet and the pial meninges. It is the consensus in the field that the leading pathology of cobblestone cortex is the pial BM disruption (Olson and Walsh, 2002). Although it is inconsistent in the literature whether radial glial endfeet are retracted or protruded at regions of defective BM, it is clear that the normal anchorage of radial glial endfeet is relevant to the presence of intact pial BM (Georges-Labouesse et al., 1998; Graus-Porta et al., 2001; Halfter et al., 2002; Beggs et al., 2003; Niewmierzycka et al., 2005; Haubst et al., 2006; Hu et al., 2007).

In this study, we demonstrate that deletion of the mouse Gpr56 gene predominantly causes protrusion of endfeet beyond the pial surface, coinciding with regions of discontinuous BM (Fig. 5B,D,F). Given the fact that GPR56 is expressed abundantly in radial glial endfeet and that it binds a putative ligand in the ECM (Figs. 7, 8), we postulate that GPR56 regulates the proper anchorage of radial glial endfeet. Consistent with the literature, our data demonstrate that intact pial BM is essential for the exis-
tence of normal anchorage of radial glial endfeet and CR cell position (Figs. 5, 6).

A new signaling pathway in pial BM organization

Studies of human cobblestone cortex and mouse models of cobblestone-like malformations have led to the identification of many molecules involved in pial BM assembly/maintenance, including ECM constituents (laminin, nidogen, and perlecan) and laminin receptors (α-dystroglycan and some integrin family members) (Georges-Labouesse et al., 1998; Costell et al., 1999; De Arcangelis et al., 1999; Graus-Porta et al., 2001; Haltier et al., 2002; Beggs et al., 2003; Niewmierzycka et al., 2005; Haubst et al., 2006; Hu et al., 2007). Deletion of the major laminin receptors, α3β1 and α6β1 integrins, results in severe disruption of pial BM and cortical lamination defects, indicating a role for laminin–integrin interactions in the maintenance of the BM and in cortical development (De Arcangelis et al., 1999; Graus-Porta et al., 2001). Here, we introduce a new receptor, GPR56, whose ligand in the pial BM is yet unknown, into this equation. It is to be determined whether GPR56 interacts with α-dystroglycan and/or integrins in regulating the same developmental process. The GPR56 signaling pathway remains mostly unknown. Recently, GPR56 has been shown to bind tissue transglutaminase, TG2 (Xu et al., 2006). However, it is not clear whether TG2 functions as a traditional ligand for GPR56. Moreover, it has been shown that GPR56 associates specifically with Gαq/11 and Gβ as part of a larger complex with tetraspanins CD9 and CD81 (Little et al., 2004). The function of this tetraspanin–GPR56 complex remains unclear. Members of the tetraspanin family of cell surface proteins act as molecular scaffolds with known adhesion proteins such as integrins to facilitate their function (Levy and Shoham, 2005). It is possible that GPR56 functions in a receptor complex with tetraspanins and integrins in regulating/maintaining pial BM integrity.

References


