

# Laminating the mammalian cortex during development: cell polarity protein function and Hippo signaling

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**During mammalian brain development, radial glial progenitors balance between proliferation and differentiation to generate the laminated cortical layers in a temporally precise fashion. Defects in the individual steps going into this complex organogenesis can result in cortical malformations and human nervous system disorders. In this issue of *Genes & Development*, Liu and colleagues (pp. 763–780) present experimental evidence that an evolutionarily conserved cellular polarity gene, *Pard3* (*partitioning-defective 3*), controls the balance of radial glial proliferation and differentiation through interaction with the Hippo signal transduction pathway. Conditional deletion of *Pard3* in the developing rodent cortex resulted in striking subcortical band heterotopia, reminiscent of a severe form of human cortical malformation.**

Cortical malformations are a form of central nervous system (CNS) disorder that has given us fundamental insights into mammalian brain development. These genetic diseases are grouped into three major categories including malformations caused by aberrant processes in (1) neuronal/glial proliferation or apoptosis, (2) neuronal migration, and (3) post-migrational development (Barkovich et al. 2012). Those cortical malformations arising from cellular migration defects are further subdivided into heterotopia, lissencephaly, subcortical band heterotopia, and cobblestone malformations. The developmentally expressed *Doublecortin* (*DCX*) gene was first identified as a commonly mutated gene in subcortical band heterotopia patients, a class of cortical malformations associated with neuronal migration defects (Ross and Walsh 2001). Depending on the type and disease severity, patient symptoms accompanying cortical malformations can range from developmental delays to motor deficits to intellectual disability to epilepsy. In this issue

of *Genes & Development*, Liu et al. (2018) describe a novel mutant mouse line with a conditional deletion of *Pard3* (*partitioning-defective 3*) that develops megalencephaly with a giant ribbon-like subcortical band heterotopia. Intriguingly, this phenotype was induced via a combination of abnormal radial glial progenitor (RGP) proliferation and untimely neuronal differentiation instead of primarily through defective neuronal migration. The Par polarity gene family consists of seven members in its core group: three kinases (aPKC, Par-1, and Par-4), two scaffolding proteins (Par-3 and Par-6), one RING finger protein (Par-2), and a member of the 14-3-3 family of proteins (Par-5) (Goldstein and Macara 2007). Originally identified in a *Caenorhabditis elegans* genetic screen for asymmetric division mutants, this protein family establishes cellular polarity in diverse tissue types and is evolutionarily conserved (Kemphues et al. 1988). In epithelia, *Pard3* is localized to tight junctions in complex with *Pard6γ* (*Pard6γ*; also known as Par-6) and *Prkci* (protein kinase C<sub>i</sub>; also known as aPKC), forming the boundaries between apical and basolateral domains. During mammalian cortical neurogenesis, RGPs retain both tight and adherens junctions to establish apical–basal polarity that is essential for their symmetric and asymmetric divisions during proliferation. While *Pard3* function has been examined previously during CNS development (Bultje et al. 2009), it has not been genetically deleted from the rodent brain in vivo.

Using an *Emx1*-cre driver (targeting the cortex) to conditionally delete *Pard3* from embryonic RGPs, the investigators reported striking subcortical band heterotopia in the cerebral hemispheres. Temporal analyses of the mutant phenotypes showed that *Pard3* plays potentially different roles during cortical lamination at two distinct developmental stages. During the early stages of RGP proliferation (embryonic days 13–15.5 [E13–E15.5]), *Pard3* conditional mutants displayed increased symmetrical divisions,

[**Keywords:** Hippo signaling; Notch signaling; megalencephaly with heterotopia; neurogenesis; *pard3*]

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Article is online at <http://www.genesdev.org/cgi/doi/10.1101/gad.316711.118>.

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leading to aberrant and excess production of RGP located outside the ventricular zone. This resulted in a concomitant reduction in the numbers of early-born SABT2<sup>+</sup> deep-layer neurons. Paradoxically, by E15.5–E17, *Pard3* conditional mutants showed an increase in SABT2<sup>+</sup> neurons just below the cortical plate as well as increased symmetrical differentiation of RGP. The investigators attributed these seemingly divergent phenotypic outcomes following *Pard3* deletion to varying levels of Notch activity between early and late cortical neurogenesis, consistent with an earlier observation from the same group showing *Pard3*'s involvement in Notch signaling (Bultje et al. 2009).

Delving deeper into the underlying molecular mechanisms, the investigators reasoned that the subcortical band heterotopia phenotype seen may be analogous to dysregulations in organ size control. The Hippo pathway is an evolutionarily conserved cellular signaling mechanism critical for regulating the physical size of multiple organ systems (Yu and Guan 2013). In a tour de force set of genetic experiments, Liu et al. (2018) conditionally removed Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), two Hippo pathway downstream effectors that remarkably were able to rescue the megalencephaly and ribbon-like heterotopia phenotypes seen in *Pard3* mutant animals. Growth factors, mechanical stimuli, polarity proteins, and morphogens intersect the Hippo pathway to control stem cell/progenitor cell proliferation, maintenance, and apoptosis (Yu and Guan 2013). A previous study of YAP during cortical development showed that its deletion resulted in hydrocephalus but without obvious defects in cortical lamination (Park et al. 2016). Similarly, the double knockout of YAP and TAZ here showed a hydrocephalic phenotype with no associated defects in cortical lamination. Although the rescued animals appeared to have reduced numbers of CUX1<sup>+</sup> cortical neurons, given that *Pard3* has additional functions in progenitor cell cycle spindle orientation and progeny migration, the extent of the in vivo phenotypic rescue via YAP/TAZ deletions remains striking. The Hippo pathway and YAP/TAZ have been linked recently to cell migration (Sakabe et al. 2017), providing a potential molecular basis for the in vivo rescue of the *Pard3* mutation phenotype. The investigators' observation that Hippo signaling was abnormally low during the early phase of cortical neurogenesis in *Pard3* conditional mutants but became induced during the late phase is also consistent with the mutant phenotypes.

This elegant and mechanistic study connects the evolutionarily conserved *Pard3* gene to megalencephaly and ribbon-like subcortical heterotopia. Human *PARD3* mutations have thus far been implicated in neural tube defects (Chen et al. 2017), but this study provides a strong rationale for further examination of its linkage to brain developmental defects. In addition to analyzing heritable germline genetic mutations in cortical malformations, screens focusing on somatic mutations arising during de-

velopment have been fruitful. Extracting DNA from blood leukocytes and using next-generation sequencing to look at specific genes known to cause cortical malformations, *DCX* and *Lissencephaly 1 (LIS1)* somatic mutations have been identified in individuals with double-cortex syndrome and periventricular nodular heterotopia, respectively (Jamuar et al. 2014). A similar screening approach for potential genetic mutations in *PARD3*, related cellular polarity genes, and components of the Hippo signaling pathway may uncover novel human defects that give rise to cortical malformations during development.

## Acknowledgments

This work was supported by National Institutes of Health grants R01MH105416, R01NS078192, and R01NS096096 and the March of Dimes.

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