Cortical Compass: *EML1* Helps Point the Way in Neuronal Migration

Mutations in *EML1* Lead to Ectopic Progenitors and Neuronal Heterotopia in Mouse and Human.

Kielar M, Tuy FP, Bizzotto S, Lebrand C, de Juan Romero C, Poirier K, Oegema R, Mancini GM, Bahi-Buisson N, Olaso R, Le Moing AG, Boutourlinsky K, Boucher D, Carpentier W, Berquin P, Deleuze JF, Belvindrah R, Borrell V, Welker E, Chelly J, Croquelois A, Francis F. *Nat Neurosci* 2014;17:923–933.

Neuronal migration disorders such as lissencephaly and subcortical band heterotopia are associated with epilepsy and intellectual disability. *DCX*, *PAFAH1B1* and *TUBA1A* are mutated in these disorders; however, corresponding mouse mutants do not show heterotopic neurons in the neocortex. In contrast, spontaneously arisen HeCo mice display this phenotype, and our study revealed that misplaced apical progenitors contribute to heterotopia formation. While HeCo neurons migrated at the same speed as wildtype, abnormally distributed dividing progenitors were found throughout the cortical wall from embryonic day 13. We identified *Eml1*, encoding a microtubule-associated protein, as the gene mutated in HeCo mice. Full-length transcripts were lacking as a result of a retrotransposon insertion in an intron. *Eml1* knockdown mimicked the HeCo progenitor phenotype and reexpression rescued it. We further found *EML1* to be mutated in ribbon-like heterotopia in humans. Our data link abnormal spindle orientations, ectopic progenitors and severe heterotopia in mouse and human.

Commentary

Defects in neuronal migration constitute a broad class of developmental disorders that lead to the ectopic localization of neurons. In many cases, deficits in cell motility, proliferation, and surrounding parenchyma have been implicated (1). The phenotype of patients with neuronal migration defects ranges from subclinical to debilitating (2). Of particular relevance for neurologists is that although cortical malformations arising from migration disorders affect only 1% of the population, the incidence rises to 14% in patients with epilepsy (3).

Subcortical band heterotopia (SBH) is often called "double cortex syndrome" (3) based on the presence of bands of ectopic gray matter within the white matter below the cortex (2). Clinically, SBH is most often caused by mutation of the gene doublecortin (*DCX*) that leads to moderate cognitive deficits and severe epilepsy (2). However, multiple etiologies in addition to *DCX* mutations also lead to SBH (1). Recently, a spontaneous mutant mouse (called the HeCo mouse for "heterotopic cortex") was identified with a phenotype consistent with SBH, including band heterotopia, developmental delay, and lowered threshold for seizures (4).

In a groundbreaking work published earlier this year in *Nature Neuroscience,* Kielar and colleagues set out to identify the gene responsible for the HeCo mouse phenotype as well as its function. The authors first characterized the neurons in the heterotopic band by colabeling the neurons at several time points for markers of early- and late-born developmental identity. Surprisingly, they noted that both early and late progenitors were present in the heterotopic gray matter (although they arrive there in a delayed fashion relative to wildtype). The insight that both early- and late-born neurons migrated to the heterotopic cortex provided the first clue that the mutation in the HeCo mice was unrelated to the capacity of the neurons to migrate.

To demonstrate directly that the migratory capacity of the HeCo neurons was intact, the authors used slice cultures and labeled neurons with GFP. By video monitoring the slices, they were able to watch the HeCo neurons initial development from progenitors as well as the subsequent migration of neurons into the cortical plate. Surprisingly, they noted that the HeCo neurons migrated at the same speed as those in the wild-type animals. This is of particular relevance, as many other genetic mutations implicated in SBH cause compromised migratory capacity (1). Interestingly, despite normal migration speed, fewer HeCo neurons successfully arrived in the cortical plate (the precursor structure to the cortex in the adult). This suggested the possibility that although the HeCo neurons themselves were capable of migration, perhaps they were inappropriately responding to extracellular cues. To examine this possibility, they transplanted labeled HeCo neurons into wild-type cultures to observe their migratory capacity. Again, they saw no difference in the rate of migration of the HeCo cells. These experiments strongly suggest that the defect in HeCo mice does not affect neuronal migration.

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A second explanation for the reduced number of cortical plate neurons in HeCo mice is that the rate of proliferation was impaired. By staining for cells that were actively dividing, the authors found that the radial glial cells (RGCs) that give rise to cortical neurons were ectopically located throughout the cortex. Interestingly, when the authors restricted their analysis to the appropriately located RGCs, they noted that these tended to be oriented inappropriately. Rather than being oriented with their cleavage plane perpendicular to the cortical plate, HeCo progenitors were more frequently oriented away from the midline. Hence, the HeCo mouse is characterized by migration-competent neurons whose progenitors are located aberrantly and oriented incorrectly.

In a tour de force of molecular genetics, Kielar et al. combined genetic linkage analysis with a microarray study to identify the echinoderm microtubule-associated protein-like 1 (*Eml1*) gene as the gene most likely mutated in HeCo mice. By sequencing mutant mouse DNA, they next determined that the HeCo mouse has a premature stop codon in exon 23 of the *Eml1* gene, leading to truncation of the final protein.

To demonstrate causality, the authors next performed a compelling series of rescue and knockdown experiments. First, they demonstrated that expressing the wild-type *Eml1* gene in the RGCs of HeCo mice was sufficient to reduce the number of ectopically located RGCs. To demonstrate necessity, they then reduced expression of the *Eml1* gene in wild-type RGCs using an siRNA strategy. Knockdown of *Eml1* expressed in otherwise healthy neurons caused the RGCs to migrate ectopically (like the RGCs in HeCo mice).

Although they had successfully shown that the HeCo mice experience SBH as a result of mutation in *Eml1,* human mutations in the homologous human gene *EML1* have not previously been reported (3). The authors thus screened a large population of patients diagnosed with heterotopias. They successfully identified two different families with distinct mutations in *EML1*. In both cases, the affected family members were found to have bilateral giant-ribbon heterotopia characterized by periventricular and ribbon-like subcortical heterotopia, polymicrogyria, and agenesis of the corpus callosum.

The authors have convincingly demonstrated a causal role for *Eml1* mutations in the HeCo mouse and subsequently demonstrated the importance of this gene in human disease. *EML1* encodes a microtubule-associated protein (MAP), which fits well into the growing literature on the importance of MAPs in the pathogenesis of heterotopias. Both *DCX* and *TUBA1A* mutations have been associated with subcortical band heterotopias, and each plays a role in microtubule based vesicle trafficking (1). More generally, *LIS1* (associated with lissencephaly) encodes a component of the dynein complex (a molecular motor related to microtubule function) (1). However, most studies examining the role of MAP mutations in heterotopias have focused on the role microtubules play in cell motility and vesicle trafficking. Here, the authors have gone to great lengths to show that despite the mutation in *Eml1* (a MAP), no such motility deficit exists. Instead, it appears to be the case that *Eml1* is important for successfully orienting the cleavage plane of neuronal progenitors in the developing brain. This deficit appears subtle, but the correct orientation of the cleavage plane during cell division is crucial for ensuring that daughter cells are positioned correctly by cell attachments (5). Although further studies are needed to understand the precise role of *Eml1* in neuronal localization, the work by Kielar and colleagues has opened up a new window into our understanding of the role of MAPs in heterotopias.

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