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## **Micro(*TUBB3*)ules Rule in Brain Development**

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### **Abstract**

**The microtubule network is crucial for the developing nervous system and mutations in tubulin-encoding genes disrupt neuronal migration. In this issue, Tischfield et al. (2010) report that mutations in the neuronal *TUBB3* gene have a striking impact on microtubule dynamics resulting in a diverse set of disease symptoms.**

The formation of the nervous system is a complex process that requires functional microtubules during all stages of development. Events such as neurogenesis, neuronal migration, axon pathfinding and synapse formation are regulated by intrinsic and extrinsic pathways that ultimately impinge on the microtubule network, which carries out the structural changes that underlie each process. However, it is only recently that human mutations have been discovered in genes encoding tubulin, the monomer that

polymerizes into microtubules. Mutations in human genes such as *TUB1A1* and *TUBB2B*, which encode  $\alpha$ -tubulin and  $\beta$ -tubulin, or in genes that regulate microtubule function such as *Lis1* and *Doublecortin (Dcx)* (des Portes et al., 1998; Gleeson et al., 1998; Jaglin et al., 2009; Keays et al., 2007; Poirier et al., 2007) give rise to disorders of brain development. Such disorders are characterized by lissencephaly (lack of brain folds) and polymicrogyria (excessive brain convolutions), which are caused principally by dysfunctional neuronal migration. In this issue of *Cell*, Tischfield et al. (2010) add to this growing literature with their report of new mutations in the human *TUBB3* gene encoding neuronal  $\beta$ III-tubulin. Using a multidisciplinary approach, they uncover *TUBB3* mutations that produce diverse clinical phenotypes including ocular motility disorder (CFEOM3). Surprisingly, this is due primarily to disrupted axon guidance and not dysfunctional neuronal migration. Furthermore, this study elegantly examines the relationship among *TUBB3* mutations, their impact on microtubule function and clinical symptoms.

Using a family-based approach, the authors identified eight heterozygous mutations in *TUBB3*. Clinically, the authors discovered that patients with the R262C mutation (the most commonly mutated residue) or the D417N mutation display hypoplasia of the ocular motor nerve and of several other nerve tracts, indicating defects in axon guidance and maintenance. Equally interesting is the observation that patients harboring different mutations in *TUBB3* display a variety of clinical diagnoses. Although most patients suffer from CFEOM3, those with R262H, E410K or D417H mutations also possess varying degrees of facial paralysis and progressive sensorimotor polyneuropathy. This led the authors to speculate that a genotype-

phenotype relationship exists, where a specific mutation is associated with a particular set of clinical syndromes. Although these classifications would be an asset for genetic counseling, they are not absolute as there are varying degrees of a given clinical manifestation. Nonetheless, how do different clinical phenotypes arise from mutations within the same gene?

To answer this, the authors turned to mouse genetics and created a knock-in model of the most common mutation, R262C. Analysis of mice with the homozygous R262C mutation in *Tubb3* revealed profound disruption of several axon tracts, including oculomotor nerves, commissural axons, branching of cranial nerves, thinning of the anterior commissure, as well as stunted growth of the corpus callosum. These data strongly suggest that the primary defect is disrupted axon growth, which agrees well with the human imaging data and demonstrates the validity of using the *Tubb3*<sup>R262C/R262C</sup> mouse as a model. Surprisingly, the authors found that the neuronal layers in the cerebral cortex were normal with no evidence of structural defects. This suggests that the R262C mutation does not affect neuronal migration, which is also consistent with the lack of cortical layering abnormalities seen in patients with TUBB3 mutations.

The authors further characterized the mutant mice and speculated that the R262C mutation disrupts the biochemical properties of  $\beta$ III-tubulin resulting in abnormal microtubule function. Indeed, the microtubules of neurons from these mutant mice have increased tyrosination, a post-translational modification that increases microtubule stability. However, simultaneously the microtubules show decreased binding to Kif21a, a kinesin motor protein. This is very interesting given that humans with heterozygous mutations in Kif21a display isolated ocular motor dysfunction with signs of axon deficits

in the cranial nerves, similar to patients with TUBB3 mutations (Yamada et al., 2003). Given that motor proteins such as the kinesins are important for transport of cargo along microtubules (De Vos et al., 2008), decreased interaction between Kif21a and microtubules might lead to defective axonal transport of growth materials and signals necessary for axon outgrowth.

So how do the different mutations produce the different clinical phenotypes given that they are all located within  $\beta$ III tubulin? To answer this, the authors performed a series of in depth biochemical and cell imaging assays to determine the effects of the different mutations on microtubule function. First, a cell-free system was used to demonstrate that some of the mutations disrupted the ability of tubulin to form heterodimers, which is a prerequisite for the polymerization of tubulin into microtubules. Second, the authors used the yeast model system to test the effects of single TUBB3 mutations and found that all human mutant yeast strains displayed some degree of resistance to pharmacologically-induced microtubule destabilization. This supports previous experiments suggesting that TUBB3 mutations increase microtubule stability. Finally, the authors turned to cell imaging of  $\alpha$ -tubulin tagged with yellow fluorescent protein (YFP) or the YFP-labeled kinesins, Kip3p and Kip2p. These experiments revealed that some of the mutations caused microtubule depolymerization rates to be less dynamic resulting in increased stability. Interestingly, imaging of YFP-Kip3p and Kip2p revealed that many of the common TUBB3 mutations (E410, D417 and R262) reduced the localization of kinesins at microtubule tips. This suggested that the mutations not only disrupt microtubule dynamics, but also perturb the interaction of

microtubules with proteins that use the microtubule system, similar to the observations in the mutant mice.

Although the Tischfield et al. study answers many questions stemming from the impact of TUBB3 mutations, many more remain unanswered. First, why do mutations in TUBB3 produce axon growth defects, whereas mutations in TUBA1A and TUBB2B primarily produce neuronal migration disorders? Based on the disease phenotype, it is likely that the functions of these genes differ during brain development; TUBB3 is possibly more important during axon guidance but dispensable for neuronal migration. As loss of TUBB3 leads to increased microtubule stability, this also implies that neuronal migration may require increased microtubule stability whereas axonal growth may require more dynamic microtubules in the growth cone. However, although the mutations produce different phenotypes, they all have the disruption of tubulin heterodimerization in common, which leads to aberrant microtubule polymerization. A second question is whether TUBB3 mutations disrupt the interaction of microtubules with tubulin-interacting proteins. The authors' data suggest that TUBB3 mutations inhibit the interaction of microtubules with kinesin motor proteins. However, as tubulin subunits must interact with molecular chaperones to fold appropriately before microtubule polymerization (Lewis et al., 1997), it is possible that TUBB3 mutations perturb the interaction of tubulin with chaperones or other tubulin modifying proteins. The answer to this question may also provide insight into why different TUBB3 mutations give rise to distinct clinical conditions, as different mutations may hinder the interaction of tubulin with distinct tubulin-interacting proteins thus disrupting different developmental events.

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**Figure 1. TUBB3 in brain development.** Human genetic studies have revealed that mutations in genes encoding tubulin, the monomer that polymerizes into microtubules,

lead to a variety of defects during brain development associated the abnormal neuronal migration and axon guidance. Mutations in *TUBB2B* lead to type I lissencephaly (smooth brain), whereas mutations in *TUBA1A* lead to polymicrogyria (convoluted brain) characterized by prematurely arrested neurons in the deeper cortical layers. Mutations in *TUBB3*, which encodes  $\beta$ III-tubulin, produce a different clinical syndrome (ocular motility disorder) primarily due to disrupted axon guidance during brain development. Although the mutations in the tubulin-encoding genes produce different disorders, all mutations eventually impair the formation of tubulin heterodimers. This would eventually result in sub-optimal microtubule dynamics during various developmental events. Patients with *TUBA1A* and *TUBB2B* mutations (grey lines) also display evidence of disrupted axon tract formation, however, it is unclear whether this is a secondary deficit due to the inhibition of neuronal migration.