Tubulin Mutations and Neurological Diseases

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Heterozygous missense mutations in genes for different tubu-lin isotypes, such as TUBA1, TUBB2B and TUBB3, as well as a homozygous splice-site mutation in TUBA8, have been associated with a wide spectrum of neurological diseases. Based on the major phenotypes observed and likely underlying cellular mechanisms in patients and animal models, these mutations can be divided into two categories: the frst shows lissencephaly and polymicrogyria (e.g., TUBA1, TUBA8, TUBB2B and a subset of TUBB3 mutations) due primarily to cortical cell migration problems; the second shows misinnervation / disinnervation in certain nerves (e.g., the other subset of the TUBB3 mutations) due mainly to aberrant guidance and maintenance of axons. Additional neurological symptoms can be found in both categories, including intellectual impairment and motor defects, ranging from mild to severe. Potential molecular mechanisms include disrupted formation of MT heterodimers; perturbed MT polymerization; altered interactions between MAPs or motor proteins (kinesin and dynein) and MTs; impaired axonal transport and changes in intrinsic dynamic properties of MTs. Some of these phenotypes are shared among different mutations at various sites on several isotypes, but others appear to segregate with specifc tubulin iso-types and may be confned to structural domains or even certain amino acid residues. Why some amino acid substitutions lead to distinct phenotypes while others exhibit more similarities is still not clear. One possibility is that different tubulin isotypes may play different roles in neuronal structures and functions during development and maturation, as well as maintenance and modeling throughout life. Four distinct functional aspects may be seen with a given mutation: (1) altered interactions with other tubulin monomers in forming heterodimers or with other tubulin dimers in forming protoflaments; (2) differential

binding to GTP/GDP and MAPs that regulate intrinsic properties of MT dynamics and stability; (3) reduced affnity for motor proteins needed to maintain axonal transport and axon guidance; (4) changed responses to environmental cues that determine MT polarity and direction of axon growth. Here, we use a subtype of TUBB3 mutations to illustrate genotype–phenotype correlations and to demonstrate some potential cellular/molecular mechanisms.

Neuron-specifc β-III tubulin (TUBB3) mutations may produce congenital fbrosis of extraocular muscles 3 (CFEOM3)

Recently, a class of TUBB3 mutations were shown to produce CFEOM3 (Tischfeld et al., 2010), which is a group of eye movement disorders caused by dysfunction of the oculomotor nerve and/or the extraocular muscles innervated by it, and can result from genetic errors in axon growth and guidance. Children suffering from CFEOM are usually affected at birth due to dominant negative mutations. The classical CFEOM symptoms (ptosis and restricted eye movements) are observed as well as additional nervous system disorders, such as peripheral axonal neuropathy, facial paralysis, or intellectual and behavioral impairments. Conventional neuroimaging reveals a spectrum of abnormalities including hypoplasia of oculomotor nerves, as well as dysgenesis of the corpus callosum, anterior commissure, and corticospinal tracts. The most common mutation, which causes relatively isolated CFEOM3, results from an R262C amino acid substitution. A $Tubb3^{R262C}$ knock-in mouse model reveals axon guidance defects of the oculomotor nerve and central axon tracts, without evidence of cortical cell migration abnormalities (Tischfeld et al., 2010). These defects suggest that aberrant axon growth and guidance might provide the cellular basis for the disorder, but what is the underlying molecular mechanism? Structural analysis showed that the mutation site for R262C is located in the loop between helix H8 and strand 7 of β-tubulin, below helices H12 and H11, which normally forms a

putative hydrogen bond with H12 through the carbonyl oxygen of residue D417. The R262C mutation would abolish this hydrogen bond, potentially affecting motor protein interactions with MTs and leading to isolated CREOM3. Several severe disease-associated TUBB3 substitutions (e.g., E410K and D417N/H) reside directly at putative kinesin interaction sites on β-tubulin. Further, kinesin-microtubule interactions were decreased both in the $Tubb3^{R262C}$ knock-in mouse model and in yeast models bearing the entire allelic series of mutations (Tischfeld & Engle, 2010). Therefore, mutations in TUBB3 may cause aberrant axon growth and maintenance by directly or indirectly altering MT interactions with other proteins, including but not necessarily limited to molecular motors in the kinesin family.

Thus, the genetics showed that tubulin mutations could be associated with a clinical neurological disease, CFEOM3. This phenotype is a sensitive indicator of errors in axon growth and guidance, providing insights into which functions of tubulin and MTs affect axon growth and guidance. The genetic studies generate basic neurochemical studies that illuminate pathogenic mechanisms as well as elucidating molecular pathways essential for axon guidance and circuit formation throughout the mammalian nervous system. In turn, these studies may lead to new therapeutic strategies, advancing the care of patients.