IOWA STATE UNIVERSITY

Department of Genetics, Development and Cell Biology

Pallavi Sinha-Roy's Ph.D. Defense

Monday, June 27, 2022 • 10 a.m. (virtual)

Link: https://iastate.webex.com/iastate/j.php?MTID=m7c0bddf99f9378e344cfd86f50685731

Major: Molecular, Cellular and Developmental Biology
Mohan Gupta Lab

Consequences of pathogenic tubulin-mutations on molecular motor proteins and their implications in tubulinopathies

Abstract: Microtubule cytoskeletal polymers play crucial roles during the various processes of neurodevelopment, such as cell division, dendrite arborization, axon specification, synapse formation, intracellular transport, and axon guidance/maintenance. Synchronization of these complex events is important for brain development and establishment of the central nervous system. The hollow polar microtubules are built from heterodimers of α/β tubulin. This pool of tubulin is typically composed of multiple α and β-tubulin isotypes, with humans possessing eight α and nine β-tubulins. In the last two decades, several missense mutations have been discovered in many of the α and β-tubulin isotypes, which result in the development of various neurodevelopmental diseases such as malformations of cortical development (MCDs), congenital fibrosis of the extraocular muscles (CFEOM), and a diverse range of other disease formations grouped under "tubulinopathies."

While MCDs are characterized by brain cortical lamination defects resulting due to alterations in neuronal migration and/or defects in neuronal progenitor cell division, CFEOM is a neurodevelopmental eye disease characterized by limited eye movement due to improper innervation by the oculomotor nerves and subsequent degeneration of extraocular muscles. These disease phenotypes can vary in severity based on amino acid residues mutated. Mutations in the molecular motor proteins, kinesins and dynein, along with dynein-related regulators, are also linked to various neurodevelopmental disease formations. Kinesins and dynein are important in the intracellular transport of essential cargoes in opposite directions on axonal microtubules for appropriate synaptic signaling and axon guidance/maintenance. Additionally, dynein is important for neuronal migration during cortical development. While some α-tubulin mutations linked to MCD formation disrupt neuronal migration and dynein activity, several β-tubulin mutations associated with CFEOM development disrupt kinesin activity and transport. Since some CFEOM-associated mutations in the kinesin, KIF21A, are known to hyperactivate kinesin activity rather than disrupt it, it is plausible that the mechanisms of CFEOM formation due to mutations in kinesins versus tubulins, are disparate. With the recent advancements in genome screening, there is a rise in the discovery of tubulinopathy mutations; however, the molecular etiology of these disease formations is largely unknown.

My dissertation is focused on dissecting the molecular consequences of several disease-associated α and β-tubulin mutations on molecular motor proteins and determining if these effects on kinesins and dynein are correlated with mutations associated with CFEOM and MCDs, respectively. Utilizing a combinatorial approach of in vivo, in vitro, live-cell imaging, and proteomic approaches combined with total internal reflection fluorescence (TIRF) microscopy, we show that similar to CFEOM mutations in KIF21A, the β-tubulin CFEOM mutation, R380C, also hyperactivates kinesins, thus unifying the two contrasting models of CFEOM formation due to mutations in β-tubulins and kinesins. Thus, hyperactivation or disruption of kinesins may impair anterograde intracellular transport and potentially lead to defects in axon guidance/maintenance and timely synaptic signaling. Using live-cell imaging of genetically modified yeast cells with fluorescently labeled microtubules and motor proteins, we show that disrupted kinesin localization on microtubules is a conserved effect due to CFEOM mutations, while kinesin localization due to α-tubulin MCD substitutions is comparable to wildtype, hence suggesting a fundamental difference between CFEOM and MCD mutations and potential mechanisms. Additionally, we show that α-tubulin MCD substitutions have a common effect of altering dynein activity, observed across mutations linked to MCDs of varying severity. Interestingly, our results reveal that similar to the effects of the β-tubulin CFEOM mutation, R380C, on kinesin activity, dynein activity is also hyperactivated by two α-tubulin MCD mutations, S419L and R390C, in yeast. Since dynein and dynein-related regulators are important for neuronal migration in the developing brain to properly form cortical layers, hyperactivation or disruption may impact the efficiency of neuronal migration and potentially result in cortical disorganization.

Overall, our results strongly suggest that alteration of kinesin and dynein activity is most likely conserved for CFEOM and MCD development, respectively, and that hyperactivated or disrupted molecular motor activities may be similarly implicated in CFEOM and MCD development due to tubulin mutations, thereby underlining the importance of homeostasis in molecular motor activities of kinesins and dynein, for overall cellular and organismal health.