Enhancing Microtubule Dynamics with Fidgetin-Like 2 Depletion

Katzenstein, Jed (School: Dobbs Ferry High School)

The enzyme Fidgetin-like 2 (FL2) suppresses cell migration by severing intracellular filaments called microtubules (MTs). Mouse studies showed that depleting FL2 using small interfering RNA (siRNA) promotes wound healing by enabling cells to enter the wound zone more quickly (Charafeddine et al., 2016). I hypothesized that FL2 (1) curbs neuronal axon growth by severing labile MTs and (2) curbs MT polymerization. I used siRNA to test the effect of FL2 depletion on neurite outgrowth in mouse neuroblastoma (N2A) cells and to analyze how FL2 depletion changes the MT array near growth cones of N2A cells and MT polymerization in human osteosarcoma (U2OS) cells. In N2A cells, the average longest neurite was 22um (29%) longer with FL2 depletion vs control. To characterize changes in the MT array of N2A and U2OS cells, I measured the tyrosinated:total tubulin ratio along N2A neurites (tyrosination is a marker of labile MTs). The ratio increased (MTs were more labile) near the growth cones of FL2-depleted neurites. In U2OS cells, I used live-imaging to track fluorescently labelled end-binding protein 1, which binds to the tips of polymerizing MTs (Hammond, et al., 2008) and makes growing MT tips appear as “comets,” useful for tracking MT growth speed and length, which increased 18% and 20%, respectively, following FL2 depletion. These results indicate that FL2 depletion increases MT polymerization and cell motility. Targeting FL2 may enhance axon regeneration after nerve injury, counteract neurodegenerative diseases such as Alzheimer’s associated with axonal MT loss, and improve healing in many other tissues.

Awards Won:
Third Award of $1,000