"Role of Neurofilaments and Mitochondria in the Pathogenic Cascade of ARSACS: Relevant Biomarkers for Therapeutic Development" - Dr. Heather Durham

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The aims of this research project are: 1) to determine if abnormalities in neurofilament metabolism play a central role in pathogenesis of ARSACS and the relationship to mitochondrial abnormalities and 2) to assess the function of different sacsin domains, and from that information, develop a therapeutic peptide to move forward in preclinical testing.

In addition to cerebellar involvement, peripheral neuropathy occurs in ARSACS. The Durham lab established an experimental model of ARSACS by culturing spinal cord and dorsal root ganglia derived from Sacs<sup>−/−</sup> mice (collaboration with the Brais lab). Gene knockout, to mimic loss of function mutations in the Sacs gene, delayed motor neuron development and subsequently produced bundling of intermediate filaments in neurons (neurofilaments). Mitochondrial abnormalities have also been described by the ARSACS group, and in our culture model, manifested as a shift to longer mitochondria and impaired transport. We determined that neurofilament bundling preceded mitochondrial abnormalities (altered shape and transport), suggesting that cytoskeletal disorganization is crucial in ARSACS pathogenesis and represents a valid biomarker for drug discovery. A comparable bundling of vimentin intermediate filaments occurs in fibroblasts derived from skin biopsies of individuals affected by ARSACS. Intermediate filaments influence mitochondrial fusion and distribution, but our studies in ARSACS fibroblasts demonstrate that the change in mitochondrial shape is not an obligate consequence of intermediate filament bundling (collaboration with the Shoubridge lab); this phenotype did not occur in fibroblasts from patients with Giant Axonal Neuropathy (GAN), another disorder of neurofilament metabolism that results in similar intermediate filament bundling.

Neurofilaments are composed of the low, medium and high molecular weight neurofilament proteins (NFL, NFM, NFH). NFH levels are increased in the nervous system of sacsin null mice. NFH and NFM are important mediators of neurofilament interactions. In Sacs<sup>−/−</sup> motor neurons, turnover and transport of NFH was substantially delayed in neurofilament bundles, but was normal (comparable to Sacs<sup>+/+</sup> cultures) in unbundled neurofilaments (measured by fluorescence recovery after photobleaching (FRAP) in neurons expressing EGFP-tagged NFH). Neurofilament bundles could, however, be resolved after their formation by knocking down NFH expression by CRISPR/Cas9 technology in a manner consistent with the slower kinetics of NFH exchange. These experiments indicate that sacsin is not required for exchange of NFH subunits in neurofilaments per se, but does, in some manner, regulate intermediate filament dynamics.

Studying the effect of sacsin's structural domains in culture models revealed that sacsin is a multifunctional chaperone with respect to neurofilament dynamics. The UBL domain of sacsin promoted degradation of NFH and NFL, but did not directly promote their assembly into a filamentous structure. The DNAJ domain did interfere with NFL/NFH assembly, and the HEPN domain promoted structural organization of filament bundles within the cell. Toxicity was observed with over-expression of the DNAJ domain and possibly full length sacsin. Studies of the sacsin domain are in progress to complete this objective. These data are providing the basis
for designing and testing therapeutic polypeptides composed of selected sacsin domains and appropriate delivery systems. We derived an active peptide from the UBL domain fused to a cell penetrating TAT sequence and showed that this peptide has the same effect in Sacs<sup>−/−</sup> and Sacs<sup>+/+</sup> motor neurons as expression of the sequence from plasmid DNA, i.e., depleting neurons of neurofilaments, including the abnormal ARSACS bundles (collaboration with the Gehring lab). The strategy going forward is to fuse an additional domain to the Ubl to regulate its activity.
