PROGRESS REPORT – RESCUING MITOCHONDRIAL DIVISION IN ARSACS WITH CIDR

SUMMARY AND AIMS

The recently characterized *SACS* KO mouse recapitulates many of the cardinal features of ARSACS, including gait abnormalities and loss of cerebellar Purkinje cells (PCs) [1, 2]. These studies also implicated mitochondrial dysfunction resulting from impaired mitochondrial division as a pathogenic mechanism. Regardless of whether dysregulated mitochondrial fission/fusion is a primary event (as suggested by the interaction between sacsin and the mitochondrial fission enzyme Drp1 [1] and just published work by the Chapple group in patient fibroblasts [3]) or occurs downstream of cytoskeletal abnormalities [2], interventions that restore healthy levels of mitochondrial division hold promise for the treatment of ARSACS. With this grant, we will 1) directly assess the contribution of mitochondrial dysfunction to neurological phenotypes in *SACS*$^{-/-}$ mice and to 2) provide proof-of-concept evidence for the therapeutic potential of drugs that stimulate mitochondrial division. To this end, we developed a novel transgenic technology, which we refer to as *c*hemically-*i*nducible *D*rp1 *r*ecruitment (CIDR), to drive mitochondrial division, thereby accelerating mitochondrial biogenesis and clearance of dysfunctional mitochondria via mitophagy. Under control of a conditional promoter for brain-region specific expression, the CIDR transgene encodes a two-component Drp1 receptor that can be activated with a dimerizing drug (**Fig. 1**). To test our therapeutic hypothesis, we will **generate mice with CIDR in cerebellar Purkinje neurons (Aim 1), cross them with *SACS*$^{-/-}$ mice, and assess dimerizer-dependent improvements in histopathology and motor coordination (Aim 2)**. Complemented by our ongoing drug discovery efforts [4], we hope that these studies will swiftly progress to mitochondria-targeted therapies for ARSACS.

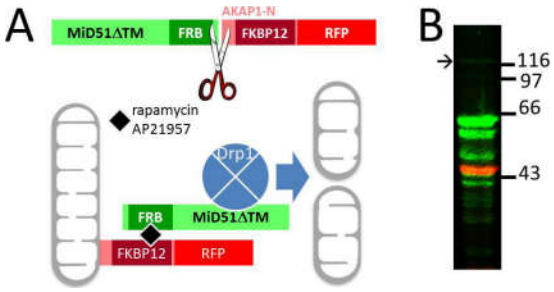

Fig. 1. Chemically-inducible Drp1 recruitment. ***A***, domain diagram of the bicistronic construct and principle of CIDR. ***B***, a CIDR plasmid with a GFP-tagged MiD51 module was expressed in HEK293 cells and total lysates were blotted for GFP (green) and mRFP (red) followed by simultaneous 2-color detection. Efficient ribosome skipping is indicated by the only faintly detectable precursor band (arrow).

PROGRESS FROM SEPTEMBER 2015 TO JUNE 2016

We generated the conditional CIDR transgene vector and tested its effectiveness by co-transfection with Cre recombinase in neuronal cultures. We then submitted the vector to our Genome Editing Facility (Bill Paradee director) for oocyte injection. So far, we obtained one founder mouse with germ line transmission of the transgene (Fig. 2). We prepared cerebellar and hippocampal neuronal cultures from the founder's offspring and infected these cultures with AAV-GFP-Cre to remove the floxed-STOP cassette and thereby turn on the transgene. Unfortunately, we did not detect transgene expression (as evidence by mRFP-labeled mitochondria) in GFP-Cre positive neurons (not shown). While our data so far suggest that the transgene may have inserted into a transcriptionally silent chromosomal region, we have set up crosses of founder #1 with Nestin-Cre expressing mice to effect recombination in vivo. In the meantime, our Genome Editing Facility continues their efforts to generate more CIDR founder mice (3 have been promised).

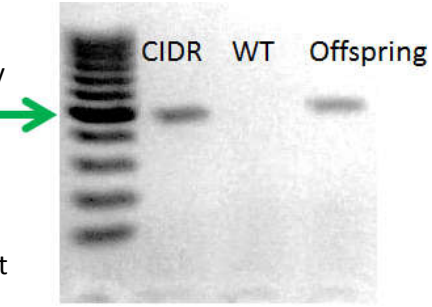

Fig. 2. Germline transmission of CIDR transgene. The genotyping PCR detects a 467 b amplicon specific for the transgene.

On a more positive note, Jennie Liu, my graduate student that works on the ARSACS project has just completed her comprehensive examination and is now able to fully devote herself to her thesis research. I am also about to make an offer to a highly qualified postdoctoral candidate, who has expressed great interest in our ARSACS work.

REFERENCES

1. Girard, M., et al., *Mitochondrial dysfunction and Purkinje cell loss in autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS).* PNAS, 2012. **109**(5): p. 1661-6.
2. Lariviere, R., et al., *Sacs knockout mice present pathophysiological defects underlying autosomal recessive spastic ataxia of Charlevoix-Saguenay.* Hum Mol Genet, 2015. **24**(3): p. 727-39.
3. Bradshaw, T.Y., et al., *A reduction in Drp1 mediated fission compromises mitochondrial health in autosomal recessive spastic ataxia of Charlevoix Saguenay.* Hum Mol Genet, 2016.
4. GSK2014DiscoveryFastTrackChallenge, *http://openinnovation.gsk.com/news-2014-12-01/*. 2014.