Regenerative Medicine Minnesota
Grant Number: RMM 11215 DS003
Principal Investigator; Jonathan Marchant, PhD
Project Timeline: 2/1/2016 – 1/31/2018

NAADP Signaling: novel pharmacotherapy for neuronal regeneration and repair

This document provides a final progress report for the RMM funding period of this project, as requested in grant award correspondence.

Overview. There is a key need to generate new drug treatments for neurodegenerative diseases that halt pathologies underpinning disease progression rather than just mask symptoms of these disorders. In this regard, analogs of nicotinic acid adenine dinucleotide phosphate NAADP - a potent Ca^{2+} releasing second messenger in many cells and tissues – have been shown to be competent at reversing cellular defects seen in cells isolated from patients with a familial Parkinson disease. To understand whether this therapy has clinical potential, we proposed to discover and optimize the properties of NAADP mimetics for use *in vivo* and assess the beneficial effects of newly generated lead compounds on neuronal differentiation, regeneration and long term viability.

Experimental Progress. Two research papers have been submitted based around the experimental findings summarized within this report. Research findings that relate to potential intellectual property have been redacted. Submitted publications resulting from RMM Support:

(1) ► A screening campaign for discovering modulators of NAADP-dependent Ca^{2+} signals in human cells. Gunaratne *et al.* (Submitted, 2018)

(2) ► Inhibition of Two-Pore Channels blocks translocation through the endolysosomal system. *Gunaratne et al.* (Submitted, 2018)

Both submitted manuscripts acknowledge support from Regenerative Medicine Minnesota (RMM 11215 DS003). Further details will be provided when studies are accepted for publication. Data were also presented as a poster presentation to attendees at the annual RMM retreat on June 1st 2018 at the Mayo Clinic. Our findings, and both associated publications, will facilitate the submission of future, extramural funding applications.

Summary of key findings:

<u>*Publication #1*</u>. *Discovery of novel chemical scaffolds that manipulate NAADP signaling system*. Ca^{2+} signals originating from the 'acidic' Ca^{2+} stores of endosomes and lysosomes regulate a steadily growing list of cellular and developmental processes. NAADP mobilizes intracellular Ca^{2+} stores by engaging the activity of members of the two-pore channel (TPC) family. TPCs are broadly expressed ion channels and evolutionarily ancient members of the voltage-gated ion channel superfamily. Their identification and subsequent study has facilitated resolution of many pathophysiology processes dependent upon endolysosomal Ca^{2+} release. It has previously been shown that NAADP analogs were competent at reversing cellular defects seen in cells isolated from patients with a familial Parkinson disease (PD), raising the prospect of developing novel analogs to treat (PD) defects and/or help facilitate the differentiation of neural stems into dopaminergic neurons. Figure 1 depicts our model that inhibition of NAADP-evoked Ca^{2+} signals, or blockade of TPCs will reverse lysosomal abnormalities seen in Parkinson's disease afflicted patients.

Owing to the intracellular localization of TPCs in lysosomes it is difficult to screen activity of analogs. We have circumvented this barrier by optimizing single cell microinjection and genetic reporter imaging methods to deliver ligands and resolve their activity at lysosomal calcium release. During the course of this grant, we completed testing ~1534 compounds in a validation pipeline for discovering novel modulators of NAADP-evoked Ca^{2+} release which resulted in the prioritization of 7 new chemical scaffolds for further analysis (Figure 2, compounds ranked by # compared to existing molecules). These compounds all block NAADP evoked Ca^{2+} signals, evidencing action as novel antagonists.

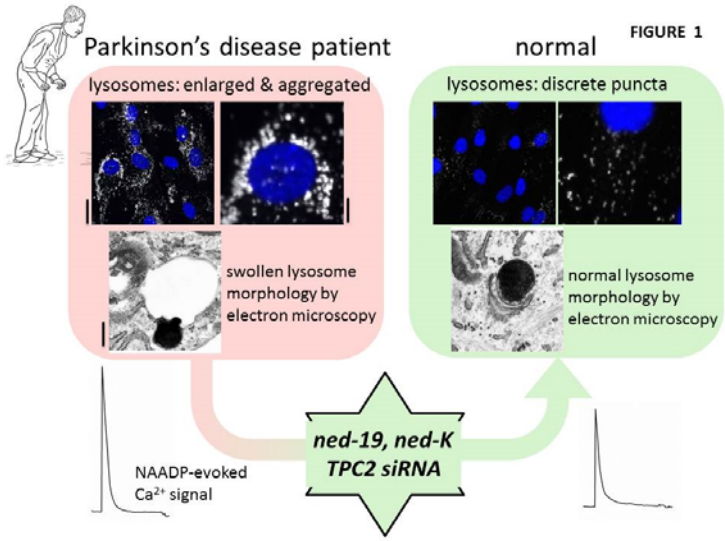

FIGURE 1. Examination of lysosomal structure and function in primary cultured fibroblasts .
Top, In healthy control fibroblasts, lysosomes were well resolved as puncta dispersed throughout the cell (right). Left, in contrast lysosomes appeared enlarged and clustered in age-matched fibroblasts derived from PD patients harboring the G2019S mutation in LRRK2. NAADP-evoked Ca^{2+} signals were also potentiated in cells from a PD patient compared with control cells (bottom). These changes in PD fibroblasts were reversed by knockdown of the endolysosomal cation channel TPC2 or by using high concentrations of NAADP blockers (ned-19, ned-k).

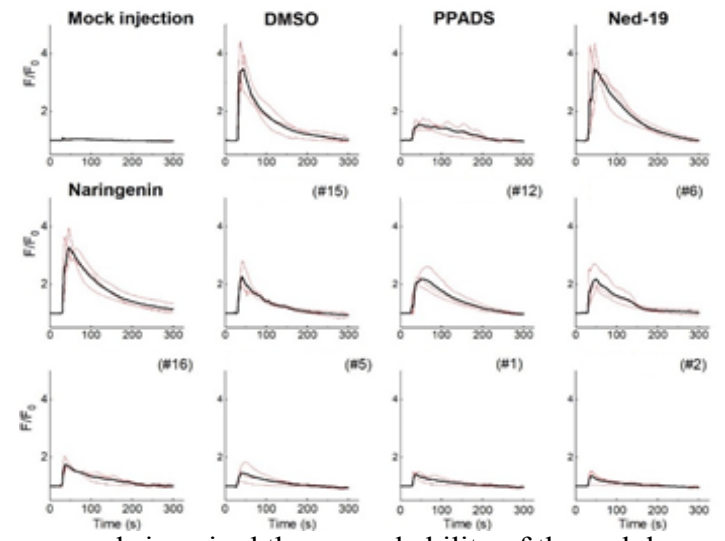

FIGURE 2. **Inhibition of NAADP-evoked Ca^{2+} release by screened drugs**. Responses to microinjected NAADP are shown in the presence of novel inhibitors (ranked by #) compared to existing modulators of NAADP-evoked Ca^{2+} signaling (ned-19, naringenin, PPADS) to illustrate improvement in TPC blockade over current modulators. Averaged responses are in black, representative traces from individual single cell imaging experiment are re shown in red. The top ranked candidates derived from a library screen (1534 compound) of known agents, therefore viable candidates have repurposing potential.

[redacted]

Publication #2. Mechanistically, we confirmed these compounds impaired the normal ability of the endolysosomal system to translocate luminal substrates, which we hypothesize may relate to blocking dysregulated function of acidic Ca^{2+} stores in scenarios of neurodegenerative disease (e.g. processing of β-amyloid, synuclein etc). In parallel, TPC inhibition (the effect of the new analogs) blocks neuronal differentiation in an iPS differentiation model. These effects relate to dysregulation of intraluminal lysosomal enzymes and changes in local lipid composition.

Budget Update: Allocated funds have been spent as per accounting proforma.

Reporting to All Minnesotans. The estimated costs of Parkinson's disease are estimated at ~$15 billion/year in the US. This cost burden will rise substantially as baby-boomers age. PD will place an increasing economic burden on the state of Minnesota in terms of nursing home costs for people living with PD, their families and local communities as it is estimated the number of patients will double by 2030. Developing new treatments is of particular relevance for Minnesota health outcomes as Minnesota is one of the 'Heartland Hub' states (NE, ND, SD, IA) which exhibit the highest incidence rates for PD in the nation. Death rates in Minnesota from PD are higher than the US average, contrasting with the outcome statistics for all other prevalent diseases in our state. Therefore, there is an urgent need for new drugs for treating PD: especially for identifying novel therapies that promote neuronal regeneration, rather than focusing on alleviating symptoms. Our work explores the druggability of a novel target pathway to yield therapies designed to prevent or reverse neuronal cell loss in PD patients.

Progress Report Compiled by Jonathan Marchant