February 5, 2015
Project update for FSMA Canada (Cure SMA)
Grant: KT1415 (Feb 1, 2014 to Jan 31, 2016) $50,000 per year
Title: The Non-SMN Mediated Benefits of The HDAC Inhibitor Trichostatin A
PI: Rashmi Kothary

We have just completed the first year of funding from FSMA Canada.

Objective: Our goal was to better understand how a small molecule (TSA) that is a global gene regulator ameliorates the disease symptoms and pathology in a mouse model of SMA.

Research Strategy: We have been studying what aspects of muscle growth and maintenance are targeted by TSA, both at the biological and molecular levels.

Significance of the Project: The proposed studies are directed towards gaining a better understanding of the mechanism of action behind the beneficial effects of TSA on our mouse model of SMA. Our results have shown that an Smn-independent mechanism is responsible for the benefits we have observed after TSA administration. This work has the potential to refine and advance therapeutic strategies for SMA and to generate additional pathways to target this devastating, yet common, genetic disease.

Summary of progress: A fully functional motor unit is composed of the motor neuron, which is an exceptionally large cell extending all the way from the spinal cord to the muscle that it innervates. The connection between the neuron and the muscle is called the neuromuscular junction. There is evidence to support that this junction might be the first site of pathological damage in SMA. This latter event might be occurring as a result of a combination of signals from a sick motor neuron and a sick muscle. Thus, motor neurons, neuromuscular junctions, and skeletal muscle are all affected upon SMN depletion in the context of SMA disease etiology, and all contribute to disease pathogenesis.

There are tremendous efforts underway in developing and testing therapeutics for SMA. These include approaches to increase the level of the SMN protein through gene therapy and through the use of small molecules. Equally of interest are the efforts at identifying approaches to repair the biological damage in SMA, without targeting SMN. We have been testing whether certain global gene regulators can provide benefit in the SMA context. In particular, we are testing the broad spectrum gene regulator, TSA, and its impact on attenuating the pathology in a mouse model of SMA.

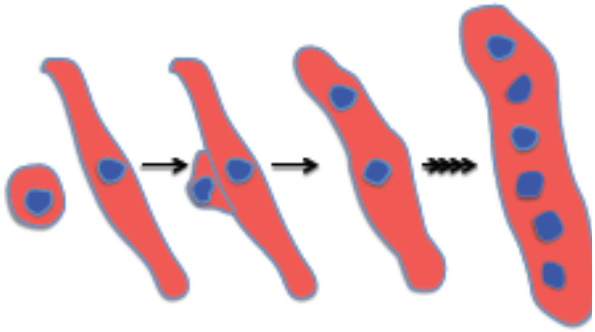
Over the past year, we have finalized experiments and published a paper showing that TSA increased the median lifespan of *Smn*$^{2B/-}$ mice (our mouse model of SMA) from twenty days to eight weeks. As well, there was a significant betterment in

weight loss and improvement in motor behavior. Furthermore, motor neurons in the spinal cord of *Smn*$^{2B/-}$ mice were protected from degeneration. Of interest, TSA did not increase Smn protein levels in the hind limb muscle, heart, or liver of *Smn*$^{2B/-}$ mice, suggesting that the benefit is coming from an effect on other protective mechanisms. As such, we predict that identification of the pathways regulated by TSA in the *Smn*$^{2B/-}$ mice could lead to the development of novel therapeutics for treating SMA.

In ongoing work in this proposal, we are continuing to explore the biological benefits of TSA in *Smn*$^{2B/-}$ mice, and in particular focusing on how muscle growth and maintenance is improved under this treatment regime. In the first year of this project, we have investigated the impact of TSA on skeletal muscle defects in *Smn*$^{2B/-}$ mice. We demonstrated that TSA can improve the fusion potential of *Smn*$^{2B/-}$ primary myoblasts. We also showed that TSA reduced the proportion of myofibers with centrally located nuclei, which indicated that the muscle was being protected from damage. Moreover, *Smn*$^{2B/-}$ mice treated with TSA had significantly healthier myofibers. TSA administration resulted in a significant decrease in the expression of embryonic and neonatal myosin heavy chain proteins suggesting that these muscles were further along the maturation process than those from untreated *Smn*$^{2B/-}$ mice. Finally, we observed a significant increase in expression of myogenic proteins following TSA administration. Other aspects of the muscle defects are being explored, such as impact of TSA on atrophy and the atrogenes, and on the proteasomal pathway, both of which are impacted in SMA.

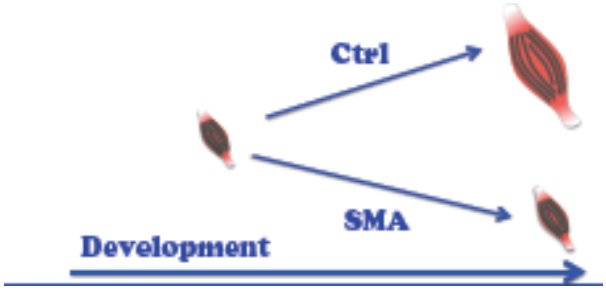
In the second part of this project, we are initiating experiments to identify gene regulatory pathways that are activated by TSA in *Smn*$^{2B/-}$ mice. It is our goal to understand what the gene activity differences are between muscles from sham treated and TSA treated *Smn*$^{2B/-}$ mice. During year one of this project, we optimized the procedures for this protocol. In the second year, we will perform the initial molecular analysis. The next stage will be to use software to compare gene activity. This work has the potential to refine and advance therapeutic strategies for SMA and to generate additional pathways to target the disease.

A



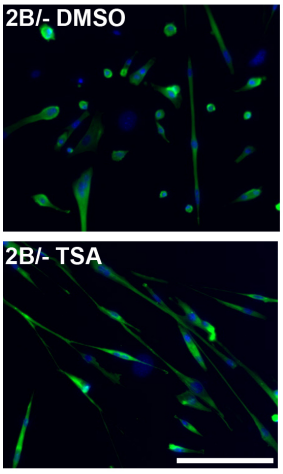
MUSCLE FORMS BY THE FUSION OF SMALL STEM CELLS TO RESULT IN LONG MYOTUBES

B



MUSCLES IN SMA DON'T GROW AS WELL

C



TSA TREATMENT IMPROVES MUSCLE CELL FUSION AND MUSCLE GROWTH IN Smn2B/- MICE