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1. Introduction and summary of previous results

Motor neurons control muscle contraction, and one of the early events in ALS is the loss of connections between these neurons and their target muscles. Growth factors secreted by the muscle control both neuromuscular connections and the survival of the motor neuron. These growth factor molecules bind to receptors on the surface of the neuron and are transported in membrane-bound endosomes to the neuronal cell body in the spinal cord, relaying a pro-survival signal. While we know that growth factor signaling is defective in ALS (Tovar et al., 2014), we still do not understand at what point dysfunction arises during the itinerary of a growth-factor bound receptor in diseased neurons.

In our last detailed annual scientific report, we described our progress in understanding how growth and survival signaling is altered ALS. We have used the fruit fly *Drosophila melanogaster* as a model for ALS, by manipulating expression of the ALS-associated gene TDP-43, which is a master regulator of many other genes (Ling et al., 2013). An important unanswered question is which TDP-43 target genes are most important in causing ALS, and what cellular processes do they control? Fruit flies with altered TDP-43 levels exhibit motor neuron degeneration, defective locomotion, and reduced lifespan, similar to ALS patients. Dr. Mugdha Deshpande, the Blazeman postdoctoral fellow, discovered that in flies that have either too much or too little TDP-43, signaling by the growth factor Bone Morphogenic Protein (BMP) is strongly reduced, and BMP receptors do not localize to the appropriate signal-permissive early endosome. Importantly, BMP signaling has previously been implicated in ALS (Katsuno et al., 2011). Together with Dr. Suzanne Paradis at Brandeis, we developed a system to study receptor signaling in mammalian neurons expressing TDP-43, to test if there are defects comparable to those we saw in the fly model. We found that growth of these mammalian neurons is severely affected when they are modified to express this ALS gene, similar to the fly model. We hypothesized that by understanding how growth and survival signals are being diverted from their normal itinerary in diseased neurons, it may be possible to develop new therapies to return these signals to the appropriate location in the neuron.

2. Research Update

We have made significant progress this year in deciphering where receptors have been shifted in the ALS model flies, and why. While we had previously discovered that growth signaling receptors were lost from an early endosome in the ALS model flies, we did not yet know to

where they had been diverted. This year, we discovered that the receptors had been shifted to a recycling endosome compartment (which is responsible for sending receptors back to the cell surface). Most importantly, genetic manipulations that could block traffic through this recycling compartment also restore BMP signaling, synapse growth and crawling in fruit fly larvae. This restoration occurred without bypassing the expression of TDP-43 or its effects on some downstream targets. This is critical because it tells us that shifting endosome traffic may be a specific and effective intervention in disease, and gives us a particular set of endosomal machinery to target (i.e., the machinery controlling recycling traffic).

One major remaining question is exactly how TDP-43 mis-expression leads to defects in receptor traffic through recycling compartments. We are working on an interesting new hypothesis that may explain the mechanism by which this occurs. We discovered that in addition to defects in receptor traffic, the ALS model flies also had increased speeds of movement of mitochondria (the energy factory of the cell), which is a hallmark sign of loss of calcium signaling in neurons (Schwarz, 2013). Previous work from other laboratories had shown that a protein that allows calcium to flow into the neuron (a voltage-gated calcium channel) was one of the targets of TDP-43 and a potential ALS intervention candidate in flies, fish and mice (Armstrong and Drapeau, 2013; Chang et al., 2014; Polymenidou et al., 2011). Therefore, we asked if restoring this calcium channel could correct the growth-signaling defect in our flies. Our preliminary evidence suggests that it does, and we are conducting experiments to fully test how this altered calcium channel accounts for problems with normal routing of growth factor receptors.

We have also made progress in our complementary mammalian system. We have found that the ability of TDP-43 to target other genes is important for its negative effects on the growth of mammalian neurons. We have tested several signaling pathways that might be altered in these ALS model neurons, giving us some clues to what specifically has gone wrong to cause loss of neuronal growth and branching. Finally, we have generated a new viral vector to manipulate TDP-43 in large numbers of neurons so that we can take an unbiased approach to identifying the variety of growth factor signals that are misregulated, so that we can specifically test if rerouting membrane traffic can correct signaling as well as neuronal growth.

3. Next steps

The results described above lead to interesting and exciting new questions that we would like to answer in our next set of experiments. How do calcium channels affect the traffic of growth factor receptors between early and recycling endosomes in the fly model? Can we identify specific calcium channels that can rescue the defects in growth of ALS model mammalian neurons, the way they do in fruit flies? Which specific growth signaling pathways cause the defects that we see in these mammalian neurons? Our experiments in the next year will begin to answer these questions. Ultimately, we also want to know if we can observe similar growth defects and rescue them in neurons in the context of an intact animal, instead of a cell culture

dish? This will be most important for testing any potential treatments targeting growth factor signaling.

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