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Blazeman Foundation for ALS Research
Research Progress Report 5/1/2015 – 5/25/2016
Avital Rodal, Brandeis University

Please find attached a progress report describing our Blazeman Foundation for ALS Research-supported work. Your generous support this year resulted in a primary research article that is currently under peer review, as well as a published review article, which can be accessed at:

https://www.researchgate.net/publication/283512138_The_Crossroads_of_Synaptic_Growth_Signaling_Membrane_Traffic_and_Neurological_Disease_Insights_from_Drosophila).

Thank you very much,

Avital Rodal

1. Overview

Growth factors secreted by the muscles 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 trafficked (in membrane-bound compartments called endosomes) to the neuronal cell body in the spinal cord, relaying pro-survival signals. Two primary lines of evidence suggest that altered traffic of neuronal growth factor receptors, leading to changes in their signaling capacity, could be a critical defect in ALS. First, growth factor signaling (in neurotrophin, insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor (VEGF) pathways) is required for motor neuron survival, and is misregulated in ALS, leading to a switch from pro-survival to pro-degenerative signaling (Gould and Oppenheim, 2010; Tovar et al., 2014). Second, mutations in components of the membrane traffic machinery are linked to multiple human neurodegenerative diseases, including ALS (Schreij et al., 2015), suggesting that intracellular transport is an important point of cellular dysfunction, and a potential target for therapeutic intervention.

2. Summary of previous results (Years 1-2)

In our last detailed annual scientific report, we described our progress on understanding how growth and survival signaling goes awry in animal models of ALS. We had found that in a fruit fly model of ALS, growth signaling was severely reduced. In this model we alter a human ALS gene called TDP-43 (Ling et al., 2013). TDP-43 regulates a vast number of downstream RNA targets in both healthy and diseased neurons, making it difficult to identify the critical perturbation(s) that lead to disease (Polymenidou et al., 2011; Sephton et al., 2011; Tollervey et al., 2011). An alternative strategy is to take a "bottom-up" approach, by defining disease-causing cellular defects downstream of TDP-43, and devising strategies to redirect these processes to a healthy state. Dr. Mugdha Deshpande, the Blazeman postdoctoral fellow, discovered that in flies that have either too much or too little TDP-43, growth receptors do not co-localize with the appropriate early or signal-permissive endosome as in the healthy flies, but instead are shifted to a signal-non-permissive recycling endosome.

Dr. Deshpande also worked with Dr. Suzanne Paradis at Brandeis to develop a system to study receptor signaling in mammalian neurons expressing TDP-43, to test if there are defects comparable to those she saw in the fly model. She found that growth of these neurons is severely affected when they are modified to express this ALS gene, similar to the fly model. 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. Together with Paradis lab graduate student Josiah Herzog, she found that the ability of TDP-43 to target other genes is very important for its negative effects on neuronal growth, and began testing a number of mammalian signaling pathways that may underlie these defects.

3. Year 3 results

Rerouting BMP receptor traffic suppresses synaptic growth defects in an ALS model: A manuscript describing the results of our work on growth factor signaling and membrane traffic in a fruit fly model of ALS has been submitted. In this manuscript, we describe the finding that growth factor signaling is reduced in ALS models, define the specific points of the membrane transport process that are disrupted, and show that rerouting membrane traffic can restore growth factor signaling, synaptic growth, and motor function. These results show where and how we can alter membrane trafficking pathways to rescue motor neuron defects in ALS, and will guide our next steps in the mammalian system.

Calcium regulation defects in *Drosophila* models of ALS: In our last report, we described the finding that mitochondria, the energy factories of the cell, exhibit pronounced transport defects in TDP-43 misexpressing neurons. In the context of ALS, defects have been observed in mitochondrial transport, fusion and fission (Cozzolino et al., 2015; Detmer and Chan, 2007; Jiang et al., 2015; Tian et al., 2015; Zhang et al., 2015). Since mitochondrial transport is regulated in response to cytoplasmic calcium levels (Schwarz, 2013), we tested if these defects are linked to cellular calcium regulatory machinery that has previously been implicated in ALS. Indeed, mitochondrial transport defects and larval crawling defects in TDP-43 animals are rescued by restoring calcium regulation. We next tested if these calcium-dependent effects might underlie some of the growth factor receptor trafficking defects we described above. Indeed, our preliminary data indicate that restoring calcium regulation partly suppresses the growth factor receptor trafficking and signaling defects in *Drosophila* models of ALS, suggesting that calcium regulation may be a critical target of TDP-43 affecting synaptic growth and function, and may lead us to valuable therapeutic targets.

Dendritic branching and calcium defects in mammalian models of ALS: We have continued to expand and validate our mammalian model of ALS in cultured neurons. Following on our previous findings that cultured neurons fail to expand normal dendrite arbors in ALS models, we have been using live imaging to determine if dendrites fail to grow or if they retract too much. Distinguishing between these possibilities will be important for defining which signaling pathways are altered. Taking off from our findings in the fruit fly, we have been exploring whether calcium and synaptic activity underlie the branching defects in ALS model neurons. Therefore, our fly and mammalian work are beginning to converge on common calcium dependent pathways that explain how cells become sick and die in ALS, and suggest specific pathways and molecular targets to halt or perhaps even reverse these defects.

4. Next steps

Our immediate goal is to finish the studies describing a role for calcium regulation in mitochondrial and growth factor receptor trafficking defects in fruit fly ALS models. Subsequently, we will focus on the mammalian studies, which will provide the next step for defining potential interventions into calcium regulation and growth factor signaling in ALS.

5. Publicity and dissemination

a. Dr. Rodal presented a poster describing Dr. Deshpande's results at a meeting entitled "Neurobiology of Drosophila" at Cold Spring Harbor laboratory (9/29/2015-10/3/2015), and at the "Lysosomes and Endocytosis" Gordon Research Conference.
b. Dr. Deshpande presented her results in a poster at the Volen Center for Complex Systems retreat (10/5/2015) and at the annual American Society for Cell Biology meeting in San Diego from 12/12-12/16.
c. Dr. Rodal gave seminar presentations about Dr. Deshpande's work, thanking the Blazeman Foundation for ALS Research, at Dartmouth Medical School, at Boston College, and at Harvard Medical School.
d. Dr. Deshpande and Dr. Rodal published a review article, thanking the Blazeman Foundation for its support.

(https://www.researchgate.net/publication/283512138_The_Crossroads_of_Synaptic_Growth_Signaling_Membrane_Traffic_and_Neurological_Disease_Insights_from_Drosophila).

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