Blazeman Foundation for ALS Research

Research Progress Report 5/1/2016 – 4/30/2017

Avital Rodal, Brandeis University

1. Introduction and summary of previous results (Years 1-3):

Motor neuron survival and growth depends on cues from the surrounding tissues, including other neurons, muscles, or support cells. This communication between neurons and other cells involves a number of growth factors that bind to receptors on the neuron and are transported into the neurons through a network of internal membrane compartments, activating growth- and survival-promoting signals. We have modeled ALS in animal systems by manipulating the expression of TDP-43, a gene linked to familial ALS. Aggregates containing TDP-43 have been reported in most cases of ALS, both sporadic and familial, indicating the role of TDP-43 in common underlying biology of the disease. In our past reports, we described how we are using this gene to create a model for ALS in fruit flies and in cultured rat neurons. Looking at the development of neuron-muscle connections in fruit-flies, we had observed defects in growth signaling at these synapses. We have used this animal model to discover the underlying biology that is defective when TDP-43 is misexpressed, with the goal of identifying relevant biological processes and molecules that would benefit from therapeutic intervention.

As stated in our last update, we published a research article summarizing our results on the effect of TDP-43 on growth signaling at the fruit-fly neuromuscular junction (NMJ) (Deshpande et al., 2016). Our main conclusions from this work are that both increased and decreases TDP-43 levels result in defects in the growth signaling at the NMJ. However, long-range signaling at the cell body doesn't appear to be perturbed. This local defect in signaling results from mislocalization of the growth receptors to a compartment that is not permissive for signaling. Rerouting the receptors away from this membrane compartment restores the signaling and results in improvement of crawling speed of fly larvae. Thus, we have identified a way to reroute receptor traffic in ALS, with the potential to correct motor neuron defects. The research paper published in the October issue of the journal Molecular Biology of the Cell (MBoC) was chosen for 'Highlights from MBoC', a selection of papers in the journal that are deemed important for the field.

2. Year 4 results:

2B: Calcium channel defects in *Drosophila* model of ALS:

A next step in our work is to understand how TDP-43 leads to growth signaling defects. As described in our previous report, we investigated one of the known targets of TDP-43, a neuron-specific calcium channel, for its role in growth signaling at the neuromuscular junction. However, our recent data suggests that restoring calcium channel levels does not rescue growth or signaling of the NMJ. This indicates that other targets of TDP-43 are involved in the perturbation of growth signaling that we have observed, and that the calcium channel is affecting a separate pathway. We are now pursuing some of these other targets, focusing on those implicated in local growth factor signaling. At the same time, since we found remarkable rescue of many defects (including crawling and long-range transport of mitochondria) by restoring calcium channel function, so we are also continuing to pursue the role of calcium channels in our mammalian models of ALS (see below).

2C: Defects in dendritic branching and calcium signaling in mammalian ALS model:

We have been investigating the mechanisms underlying the defects in growth and branching of mammalian neurons caused by TDP-43 misregulation, in collaboration with Dr. Suzanne Paradis and Josiah Herzog, a graduate student the Paradis Lab. Using different versions of TDP-43 protein where its RNA binding domain is either modified or truncated, we have established that this defect depends on the ability of TDP-43 to bind its RNA targets. We were also able to knock down TDP-43 levels in cultured neurons using a previously validated shRNA tool (Schwenk et al., 2016). Lowering TDP-43 expression resulted in defects in dendritic branching similar to those generated by

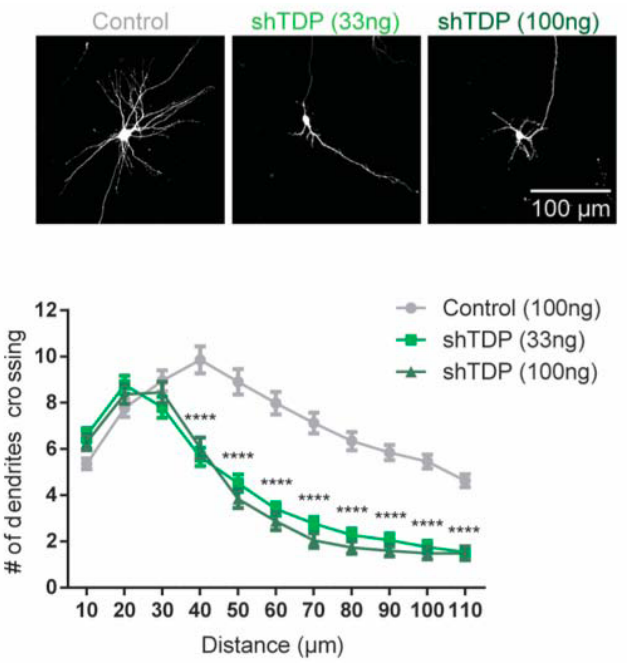

Figure 1. TDP-43 knockdown has a similar detrimental effect on neuronal branching to the defect we previously showed for TDP-43 overexpression

elevated levels of TDP-43 (Figure 1). Reminiscent of our results from the fruit fly NMJs, these data suggest that both up and downregulation of TDP-43 protein levels can lead to similar phenotypes. We also used live imaging to show that the defect is likely to occur at the step of neurite elongation, rather than neurite shrinking or retraction (Figure 2). We will submit a manuscript describing these results this month, setting the stage for defining what TDP-43 targets control neuronal branching.

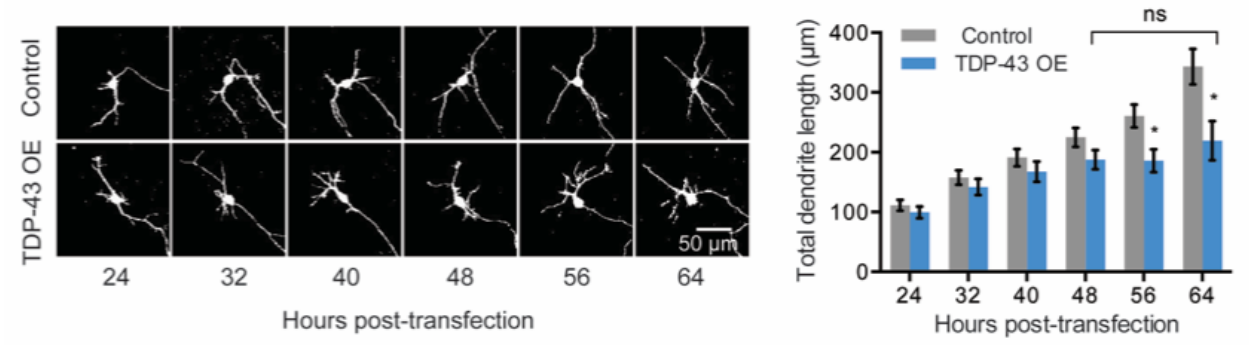

Figure 2. TDP-43 misexpression inhibits neurite elongation

Inspired by our results from the fruit fly, we had started exploring the involvement of calcium channels and neuronal activity in the context of this dendritic branching phenotype in our cultured neuron model. Our initial observations showed that neurons that overexpress TDP-43 fail to expand their dendritic arbors in response to neuronal firing. We had also observed that calcium entry into the neuron following activation is impaired in neurons the overexpress TDP-43 (Figure 3). CREB is a transcription factor that is a well-established master regulator of neuronal branching in response to activity. Our recent data indicates that in neurons that have excess of TDP-43 protein, the ability of CREB to regulate expression of other genes is impaired. We are currently testing the idea that reduced levels of calcium entering the cell upon activation lead to this impairment of CREB activation in the cells overexpressing TDP-43.

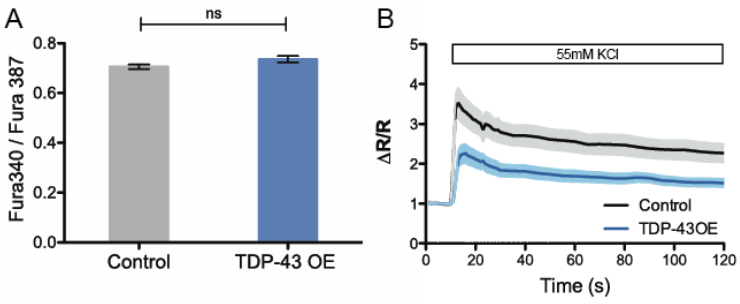

Figure 3. (A) Overexpression of TDP-43 does not change in resting calcium levels measured as a ratio of Fura-2 intensities. (B) Depolarization-induced calcium influx following exposure to 55mM KCl is significantly lowered by TDP-43 overexpression.

3. Next Steps:

We are planning to focus our future studies on exploring the molecular mechanisms underlying the defects in dendritic branching caused by TDP-43 misregulation. Although we failed to see a rescue of the growth signaling defects in the fruit fly model by restoring calcium channels, our current data suggests that in mammalian neurons, TDP-43 misregulation can lead to defects in calcium entry. We are therefore exploring the involvement of calcium signaling pathway in bringing about the dendritic branching defect. Following the idea that any diversion from optimal TDP-43 levels is equally damaging to neurons, we intend to test these pathways in TDP-43 overexpression as well as loss of function models in parallel. Dr. Deshpande is currently building experimental tools to enable these experiments.

The important next question to address is, how relevant these pathways are for progression of cellular pathology in human patients, whether they are perturbed similarly and result in equivalent cellular defects. A powerful cutting-edge technique that allows this investigation is reprograming stem cells derived from ALS patients (harboring mutations in TDP-43) to generate neuronal cell lines. We have obtained a small grant ($19,000) from Brandeis to initiate a collaboration with the Human Neuron Differentiation Core (HNDC) at Boston Children's hospital, a for-fee facility that specializes in creating such patient-derived neuronal cell lines and provides access to state-of-the-art facilities for imaging and recording neuronal activity. This will enable us to compare the ALS patient neurons to control cell lines and probe for signs of neuronal dysfunction. We are working with the Brandeis publicity office on an article describing this next step in our ALS research, which we will send to the Foundation for approval, and will credit the Blazeman Foundation for ongoing support of Dr. Deshpande in this work.

Our goals for the next 12 months are:

1. Understanding how reduced local growth factor signaling arises when TDP-43 is altered in fruit fly neurons.
2. Testing the whether the defect in dendritic branching resulting from misregulation of TDP-43 depends on the molecular pathway that leads to calcium-dependent activation of CREB.

3. Figuring out which specific calcium channels targeted by TDP-43 are involved in this pathway.
4. Checking if dendritic branching defects that arise from the loss of TDP-43 protein involve the same calcium signaling pathway.
5. Exploring the effects of TDP-43 misregulation in reprogrammed neuronal cell lines derived from ALS patients. Until now, all of our studies have been conducted in animal models of ALS, which are powerful platforms for discovery but always have the concern that the biology is not the same as a human ALS patient. Patient-derived cells provide a powerful human model in which to test our animal findings.

4. Budget

Please see attached budget summary. There were no funds spent other than paying for Dr. Deshpande's salary and benefits. A balance of $ 9,202.11 remains from carryover from 2015/2016, which can either be applied to next year's salary/benefits, or to meeting travel or project-relevant supplies going forward (in which case we can provide a letter to request that these funds be redirected to meeting attendance, publication costs, and supplies).

5. Publicity and dissemination

a. Recent findings from the *Drosophila* ALS model studies were published in a research article in *Molecular Biology of the Cell*. (http://www.molbiolcell.org/content/27/19/2898.long).
b. Dr. Deshpande presented her results in a poster at the Cold Spring Harbor Meeting "Neurodegenerative Diseases: Biology & Theraputics" (11/3/16 – 12/3/16).
c. Josiah Herzog presented a poster describing his and Dr. Deshpande's results at the Gordon Research Conference titled, "Neurobiology of Brain Disorders" (Aug. 7-12, 2016).
d. Dr. Deshpande and Dr. Rodal published an invited review article highlighting the quality and impact of a paper they reviewed for Journal of Cell Biology, thanking the Blazeman Foundation for its support. (Deshpande and Rodal, 2016) (http://jcb.rupress.org/content/214/6/641.long)

6. References

Deshpande, M., Z. Feiger, A.K. Shilton, C.C. Luo, E. Silverman, and A.A. Rodal. 2016. Role of BMP receptor traffic in synaptic growth defects in an ALS model. *Mol Biol Cell*. 27:2898-2910.

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