Wes sets a new pace for Alzheimer's research at the University of Texas Health Science Center

"The rapid, reproducible results on small amounts of tissue/cell lysates have allowed me to generate a more thorough data set on additional proteins, samples, conditions and brain regions all in the same amount, or even less time, as traditional Western blot. This technology has made it possible for small research labs to compete with the pace that research is conducted in large labs or companies."

— Miranda Orr, Ph.D., Postdoctoral Fellow, University of Texas Health Science Center

Fighting to keep our wits

Miranda Orr is a Postdoctoral Fellow at the Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center in San Antonio, where researchers study the basic biology of aging. They use animal models from invertebrates to non-human primates to study the cellular and molecular driving forces responsible for "aging". They also test the utility of pharmacological, dietary, and physical interventions to extend the healthy years of life.

Miranda's research interest centers on understanding the pathophysiology of Alzheimer's disease and the mechanisms governing the divergence of normal brain aging toward a progressive, irreversible pathogenic state. Although she's always been innately curious about brain function and aging, her research is extremely personal too. She pursued a Ph.D. in neuroscience after watching her vibrant and quick-witted grandmother slowly lose her memories after being diagnosed with Alzheimer's disease. Her goal? Make a big difference in the lives of the aging by positively influencing Alzheimer's disease research.

All hands-on, all the time

Miranda studies how age-associated changes in normal physiology alter the expression and function of the microtubule associated protein tau. Tau is highly expressed in neurons and its misprocessing occurs in many neurodegenerative disorders, including Alzheimer's disease. Tau phosphorylation negatively regulates its microtubule binding function, and sustained high levels of phosphorylation can lead to a toxic gain of function, neuronal dysfunction and degeneration.

Traditional Western blot proved to be more than a hefty task given she was comparing multiple brain regions across different experimental changes like drug intervention and dietary change, and across lifespan, and her protein of interest (tau) can gain extensive post-translational modifications at dozens of unique sites. She had all the latest equipment for high throughput Western blotting like pre-cast gels, 7-minute transfer methods, IR-dye conjugated secondary antibodies for multiplexing and a high-end Imaging system. But even with all that she felt like a processing slave with all the hands-on time. And after all the data were collected, she still had to tackle analysis, which was just as time-consuming. On top of that, low abundant proteins or small brain sub-regions were limiting how many data points she could get from any particular sample.

Clearing the smear

Wishing for a "simpler" Western, Miranda tried Wes. He let her run 24 independent samples and get fully analyzed data in about 4 hours. She also multiplexed up to 5 antibodies per capillary to get 120 independent data points in those same 4 hours. She found Wes reproducible, reliable,

fast, and used 95% less tissue and antibody. He also freed up her time for other experiments. So she started testing more proteins along with additional samples, experimental conditions and brain regions, and did it all in the same time it would take with traditional Western blots or less!

The lab's newly upped capacity also helped Miranda discover a novel high molecular weight isoform of tau protein that is expressed in the brains of the naked mole-rat (NMR), the longest living rodent known. She was perplexed by a higher molecular weight protein smear she kept seeing in her blots. But when she ran the same samples on Wes, she saw discrete high molecular weight bands much larger in size than any other brain tau isoform she'd studied before. The results showed that tau undergoes a progressive shift in molecular weight during the first year of NMR brain development (**Figure 1**, M.E. Orr et al, Neurobiology of Aging 36, 2015).

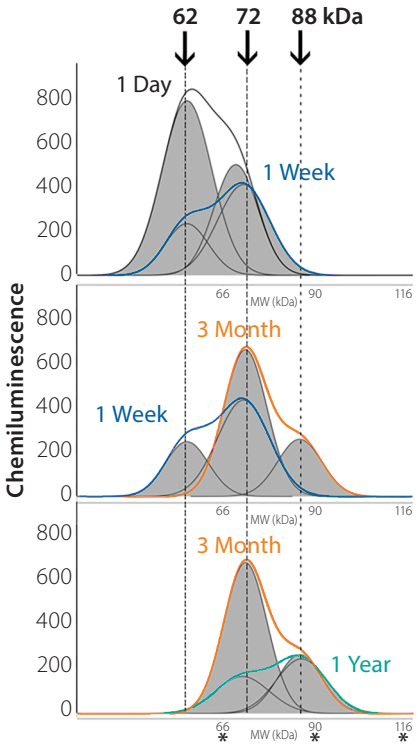

Figure 1: Detection of tau in naked-mole rats (NMR) in different stages of life development using Wes. A progressive shift in NMR tau is observed during development. Neonatal NMR tau exhibits high levels of a 62 kDa form of tau and lower levels of a 72 kDa form. During the first week of life, the tau molecular weight shifts, so the 72 kDa form is the most prominent, and an 88 kDa form emerges. At the one year mark, this 88 kDa tau protein is the most prominent form. (HT7 antibody recognizes tau at an epitope corresponding to human tau 159-163).

More time for projects

Miranda has since changed labs and started a new project with a large cell culture component. She feels Simple Western is perfect for quickly and efficiently assessing multiple treatment groups in samples with low protein yield. And she's able to examine all proteins of interest and not worry about exhausting her cell lysate supply!

Because Wes gives her time for other research now, she's also started collaborating with a laboratory using Drosophila as a model organism and is excited to assess protein expression in the brains of individual flies - research she feels is ideally suited for Wes too! More free time also means she can spend more time out of the lab with her family and friends, going to country music concerts, playing the piano and hiking with her dogs.

A few recent publications

Sustained high levels of neuroprotective, high molecular weight, phosphorylated tau in the longest-lived rodent,ME Orr, VR Garbarino, A Salinas, R Buffenstein, *Neurobiology of Aging,* March 2015; 36(3):1496-1504, doi: 10.1016/j. neurobiolaging.

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