Big hunt for small molecule to treat neurodegenerative diseases

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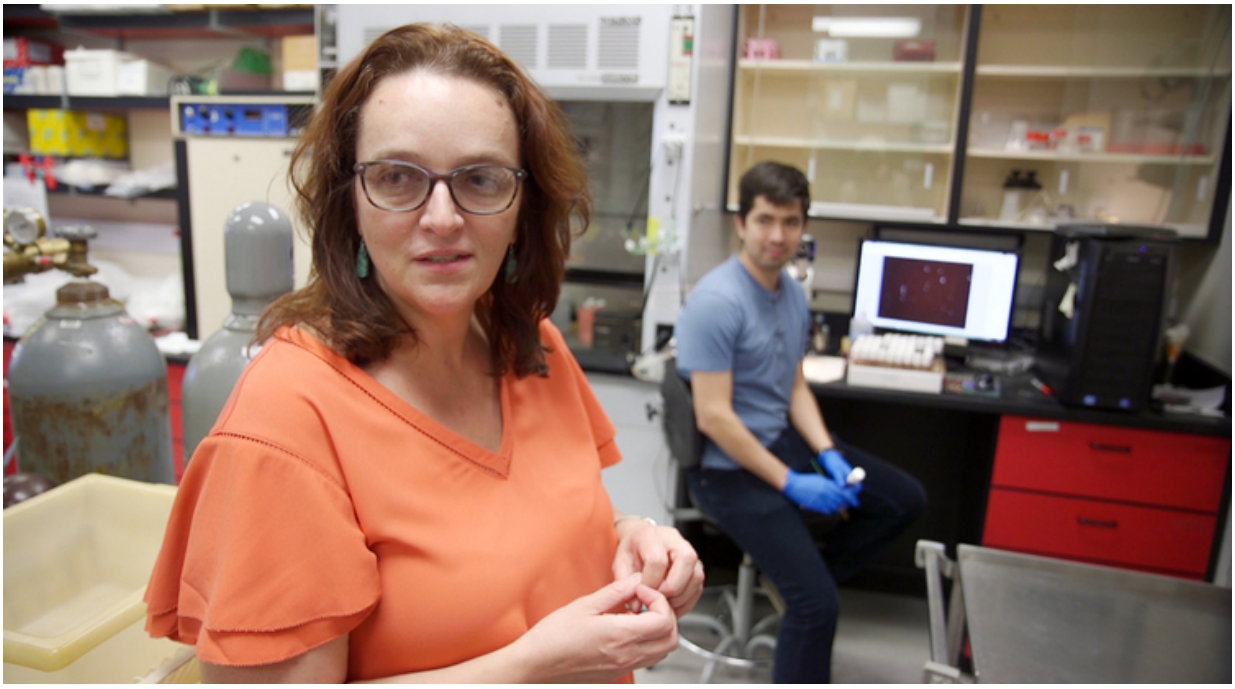

Daniela Zarnescu: "Flies make a great model that has been used to study development. Their genes are incredibly similar to humans'. About 70 percent of human disease genes are conserved in the fly genome." Credit: Bob Demers/UANews

Late last year, University of Arizona colleagues Daniela Zarnescu and May Khanna sped west on Interstate 8 bound for a brain-research conference in San Diego. Khanna is a biochemist who works with small

molecules. Zarnescu is a molecular and cellular biologist who works with flies.

"I was talking with Daniela on the way to the meeting," Khanna recalls, "and I said, 'Daniela, I want a target that's never been done before. We're coming up with a small molecule that you can test on your flies.'"

That small molecule Khanna was referring to would bind with TDP-43, a protein that has been implicated in the development of neurodegenerative diseases such as amyotrophic lateral sclerosis, or ALS—Zarnescu's and Khanna's focus. That binding, in turn, could open the door to therapeutic drugs to treat the disease.

However, finding the optimal small molecule, one that would bind with TDP-43 and that would be a safe and effective treatment for ALS, is a tall order. It's not impossible to fill, thanks to advances in genomic sequencing, the elegance and simplicity of the fly model, and the power of supercomputers.

"Flies make a great model that has been used to study development and, in the past several years, has been used to study human diseases," says Zarnescu, associate professor of cellular and molecular biology. "Their genes are incredibly similar to humans'. About 70 percent of human disease genes are conserved in the fly genome."

About 10 percent of ALS cases are inherited, or familial. The other 90 percent are known as sporadic cases. Familial ALS has helped scientists study a pedigree; that is, it has helped researchers identify familial mutations. Many genes linked to ALS have been identified that way, Zarnescu says.

"But what is interesting is that in some of the sporadic cases, you also find similar mutations, so there is a clear genetic basis to the disease,"

she says.

To complicate matters, researchers suspect that environmental factors, such as cigarette smoke and toxic chemicals, contribute to the development of neurodegenerative diseases.

The Common Denominator

No matter, the presence of TDP-43 seems to be a common denominator in most if not all people with ALS. That is, TDP-43 is found within clumps of proteins, known as cellular aggregates, which form in the brain cells of those with ALS.

"If you take postmortem samples from people with ALS, and if you look at what's in those cellular aggregates, you find TDP-43-positive aggregates in 9 percent of patients," Zarnescu says. "I call that significant."

In a normal situation, the majority of the protein resides in the nucleus, where it takes care of gene expression, Zarnescu explains. But in disease states, it emerges from the nucleus and forms cytoplasmic aggregates.

"These are a hallmark of neurodegeneration, whether it's ALS, dementia, or Alzheimer's or Parkinson's," Zarnescu says.

"Essentially, proteins aggregate and form these huge areas in cells that act like kitchen sinks where other things go and then stick. It's like a huge traffic jam, a huge clog. They're essentially altering gene expression and protein function in a very dramatic way."

At first glance, it might seem that researchers would be trying to eliminate these aggregates, but studies show that these aggregates may be protective at some stage as they are originating from what are known as

RNA stress granules.

These stress granules, composed of proteins and RNAs, form in the cells' cytoplasm when cells are under stress, whether the stress is environmental or caused by mutations, Zarnescu says.

Although their exact functions are not yet fully understood, Zarnescu explains that the granules won't allow certain RNA molecules to be translated into proteins, their final fate.

"If the stress goes away, then these RNA granules dissolve, and the RNAs are freed, and the translation starts over again," Zarnescu says. "It's like a pause for protection."

If the stress doesn't go away, the aggregates persist and they undergo an evolutionary process, she says. That evolutionary process leads to the accumulation of protein aggregates seen after death.

"This is the process that many of us are trying to understand—and what May and I are working on together," Zarnescu says. "We don't want to get rid of these aggregates because they are protective in the initial stage. This is a physiological mechanism that we need to survive."

Finding the molecule

That is why Khanna, an assistant professor of pharmacology, and Zarnescu want to find that small molecule—a molecule that will remodel the aggregates before they get out of control. "You want to release the RNAs, you don't want to sequester them forever because that's how you lead to changes in gene expression and eventually motor neuron death," Zarnescu says.

But how does one find such a small molecule?

"I drove back from San Diego by myself with no music, nothing, and I started to think about the problem," says Khanna, an assistant professor of pharmacology.

Khanna says she knew solving the problem would start with harnessing tremendous computing power. To come up with just the right molecule, she would need to sort through more than 1 million target molecules in silico. She also knew she would need Zarnescu's impeccable fly model and other collaborators, including Vijay Gokhale, UA director of Computer Aided Drug Discovery, and her students, who would help select those target molecules while learning about drug discovery firsthand.

"May is actually teaching us how small molecules bind to TDP-43," Zarnescu says. "She goes to her supercomputer and says, 'Here's a good molecule.' We find something in the fly, go back to her, and tell her what proteins and RNA we might be thinking about. She does her structural magic, and then she goes to the supercomputer. The supercomputer tells her what molecules might be even better, and then we come back to the fly and test, and we're going to go through a few iterations like that."

Will Khanna recognize a sound candidate when she sees one?

"The way I think of proteins is mountains and valleys," Khanna says. "These are like the pockets in proteins, and you want the small molecule to fit in there. That's the docking. We want them to prohibit other things to bind to them.

"The little molecule needs to fit on this groove or pocket and not allow something else to come in and dock there. You need to hit important regions. These regions you're hitting is like Achilles' heel. You hit his heel with an arrow and you kill him. You hit these regions and you

inhibit these reactions."

Provided by University of Arizona