

NEW CLUES TO UNDERSTANDING HUNTINGTON DISEASE: INCLUSION BODIES MAY PROTECT NEURONS

Carroll, Linda

Using a robotic microscope to approximate time-lapse photography, researchers have found evidence that inclusion bodies, one of the hallmarks of Huntington disease, may actually protect neurons rather than damage them.

In the new study, published in *Nature* (2004;431:805-810), investigators found that neurons containing inclusion bodies lived longer than those that lacked the clumps of mutant protein. The finding may help resolve a longstanding debate about the role of inclusion bodies, which are found in the neurons of patients with Huntington disease and several other neurodegenerative diseases.

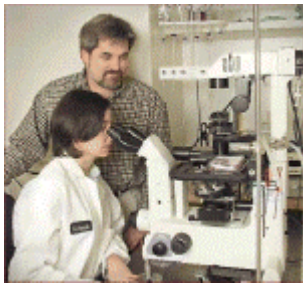


Figure. Dr. Steven Finkbeiner is pictured here with lead author Montserrat Arrasate with the robotic microscope.

They have argued eloquently that there may be a benefit to pulling disseminated huntingtin aggregates into a clump, said John Trojanowski, MD, PhD, Director of the Institute for Aging and the Alzheimer's Center at the University of Pennsylvania School of Medicine in Philadelphia.

And it is important to answer this question, Dr. Trojanowski said. Right now, no one knows whether drugs should be designed to attack the inclusion bodies or the bits of misfolded proteins that are scattered throughout affected cells, he explained.

WORK OF ROBOTIC MICROSCOPE

To take a closer look at the role of inclusion bodies, researchers from the University of California-San Francisco (UCSF) built a special robotic microscope that could follow the lives of hundreds of thousands of neurons transfected with huntingtin.

The robotic microscope allows you to go back and look at the same neurons even after the cells have been in and out of an incubator, said Steven Finkbeiner, MD, PhD, Assistant Professor of Neurology and Physiology at UCSF.

The robot keeps track of individual neurons by figuring out where they are with respect to marks on the culture dish, Dr. Finkbeiner explained. And this means you can evaluate a large quantity of neurons - 300,000 to a million - in about 15 minutes, Dr. Finkbeiner said.

The researchers also used a type of statistics - survival analysis - to evaluate risk factors for neuronal death. One way to look at this is to think of each neuron as an individual patient. We are following them throughout their lifetimes and figuring out which risk factors in the neurons predict death.

The robotic microscope allowed the researchers to chronicle, in detail, the course of each neuron. We could watch the huntingtin protein aggregate into inclusion bodies, Dr. Finkbeiner said. We could watch when neurons eventually died.

WHAT HAPPENED WITH NEURONS

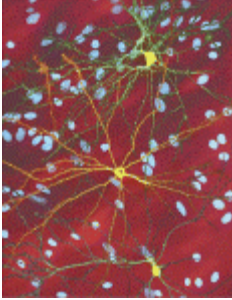


Figure. The image shows a neuron in yellow that contains the disease-associated version of huntingtin, seen as the orange-red structure in the center of the cell.

The researchers saw that when the polyglutamine structures were below a certain threshold, the neurons didn't die. They noticed that the risk of death went up as the length of the trinucleotide (or polyQ) stretch increased, Dr. Finkbeiner said.

It was just like what you see in humans, Dr. Finkbeiner said. If the stretches were less than 35Q, the neurons were fine. Above that, they survived less well. The longer the stretch, the more rapidly they died.

The researchers also noticed that neurons died even if they had no inclusion bodies. The original model suggested that inclusion bodies were directly responsible for killing the neuron, Dr. Finkbeiner said. That establishes that the diffuse form of huntingtin protein must be toxic, independent of inclusion bodies.

When the researchers looked at inclusion body formation as a risk factor for cell death, they discovered something interesting.

We found that the risk of death in a neuron that formed an inclusion body fell pretty dramatically, Dr. Finkbeiner said. This suggests that the inclusion body is a coping response.

In fact, Dr. Finkbeiner said, levels of diffuse huntingtin immediately started dropping when an inclusion body formed. By 24 hours they were 10 percent of what they had been. And in two days, you couldn't detect any diffuse huntingtin.

NOT A CURE YET

That doesn't mean that inclusion bodies fix everything, Dr. Finkbeiner said. On average, neurons with inclusion bodies live longer than those that don't mount this response, he added. That doesn't mean it's a cure.

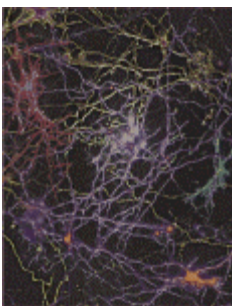


Figure. This shows a montage of four images of the development of a single neuron over a two-week period. The neuron was transfected with green fluorescent protein and an automated microscope was used to image the neurons at different intervals - from three hours (blue-green) to 137 hours (purple-gold) after transfection.

The new findings may have an impact on drug research, Dr. Finkbeiner said. People have invested a lot of money setting up drug screens aimed at trying to get rid of inclusion bodies. Some have turned up drugs that are useful, but I'm not sure they are working the way people think they are.

This opens up the possibility that we might need to think about targeting something earlier in the process. This may push the field into trying to better understand and identify what the toxic species really is.

Dr. Trojanowski agreed. There is a group of disorders characterized by misfolded proteins and inclusion bodies, he said. And nobody knows what the most toxic species is in any of them, Dr. Trojanowski added.

Is it the original molecule, or the multiples, or the protofilaments? he asked.

George R. Jackson, MD, PhD, said the study was very well done. It's innovative, and it's provocative, said Dr. Jackson, Assistant Professor of Neurology at the University of California-Los Angeles. It addresses the relationship between inclusion body formation and cell death in a way that has never been done before.

QUESTIONS ABOUT METHODS USED

Still, Dr. Jackson said, it's not the final word. The new study suggests that inclusion bodies may be protective, Dr. Jackson said. But there is a possibility that the methods used have muddied the water, he added.

That's a pretty big chunk of jellyfish protein that was fused to the huntingtin protein, Dr. Jackson said. It hasn't been proved that this doesn't alter the gene to which it is attached.

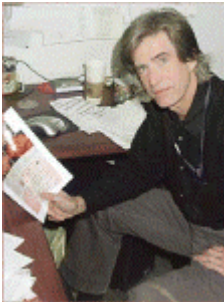


Figure. Dr. John Trojanowski said no one knows whether drugs should be designed to attack the inclusion bodies or the bits of misfolded proteins that are scattered throughout affected cells.

Dr. Finkbeiner was confident that the green fluorescent protein (GFP) did not interfere with the conclusions.

It's true that the Heisenberg principle, which says that you can't observe something without having an impact on it, applies on some level to all science, Dr. Finkbeiner allowed. But, he added, even though GFP may have changed things in subtle ways, the overall relationships are the same, so the conclusions shouldn't be affected.

What we can say for sure is that the attachment - or melding - of GFP to huntingtin did not affect its ability to recapitulate key features of Huntington disease, Dr. Finkbeiner said. For example, it did not prevent huntingtin from killing neurons in a fashion that was strictly dependent on the length of the polyglutamine stretch, as it does in Huntington disease. Second, the pattern of cell death was similar to what has been seen with Huntington disease and follows a 'one-hit' model of neurodegeneration. This pattern is impossible to measure in Huntington disease patients in the way we did, but one team of researchers performed cell counts on a series of pathological specimens and found the same pattern we did [*Nature* 2000;406:195-199].

Beyond this, Dr. Finkbeiner said, only versions of huntingtin with disease-associated polyglutamine expansions formed inclusion bodies, which is what you see in Huntington disease. And, just as

they do in Huntington patients, these inclusion bodies formed in the nucleus, perinuclear area, and dendrites.

One more bit of evidence was cited by Dr. Finkbeiner: the size of the inclusion bodies that form with versions of huntingtin attached to GFP overlap with the size of inclusion bodies found in pathologic specimens from Huntington disease.

Dr. Finkbeiner suspects that the new results may apply not only to Huntington disease, but also to other diseases characterized by neuronal inclusion bodies.

I hope this will foster interest in the question of why inclusion body formation is beneficial, he said. If we knew that, it might provide a strategy for all the other diseases that show inclusion bodies. If it turns out to be some sort of common coping response, perhaps we could tap into that and it might be beneficial in a broader way.

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