MedicalXpress

Gladstone scientists identify role of key protein in ALS and frontotemporal dementia

January 12 2010

Scientists at the Gladstone Institute of Neurological Disease (GIND) have identified the reason a key protein plays a major role in two neurodegenerative diseases. In the current edition of the *Journal of Neuroscience,* researchers in the laboratory of GIND Associate Director Steven Finkbeiner, MD, PhD have found how the protein TDP-43 may cause the neurodegeneration associated with amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitin-positive inclusion bodies (FTLDu). TDP-43, is the major component of protein aggregates in patients with these diseases. Mutations in the TDP-43 gene are also associated with familial forms of ALS and FTLDu.

"TDP-43 is a very exciting protein. We found that its location in the cell is a good indicator of the damage it may cause," said Finkbeiner, senior investigator and senior author on the study. "Our findings and our experimental model will allow further studies of this protein and how it results in disease."

Under normal circumstances, TDP-43 is a common protein that stays mostly in the nucleus. It has several beneficial functions, including binding DNA and RNA, inhibiting retroviruses, and helping with RNA splicing and nuclear body formation. It also shuttles mRNA to the cytoplasm.

However, in patients with ALS and FTLDu, TDP-43 is redistributed from the nucleus to the cytoplasm and forms insoluble TDP-43

aggregates in the nucleus, cytoplasm, or neuronal processes.

The Finkbeiner team developed a model system to find out how TDP-43 might be involved in neurodegenerative diseases. They used genetic engineering to add a fluorescent tag to normal or wildtype and mutant TDP-43 in rat neurons. The tag allowed them to easily see the intracellular location of the protein.

To determine the effects of the mutant protein, the researchers used an automated microscope that can examine hundreds of thousands of neurons individually over several days. With this large amount of data, they could use sophisticated statistical analyses to follow the fate of each individual neuron and determine its risk of death at any given time.

Their experimental system used primary neurons. These neurons are taken directly from an animal to a culture dish and provide the best cells for experiments because they retain many of the features of cells in the intact brain. In fact, Dr. Finkbeiner's system showed many "normal" features of TDP-43 in neurons. For example, wildtype TDP-43 was found in the nucleus in healthy neurons. Mutant TDP-43 was also found in the nucleus, but there was more of the protein in the cytoplasm.

Several neurons developed aggregates of the protein called inclusion bodies, which are often found in diseased neurons. In addition, the system can be easily manipulated by the investigators, making it a valuable tool for dissecting the biological mechanisms underlying diseases associated with TDP-43 deposition.

"We expect this system to be very helpful to other investigators," explained Finkbeiner.

The researchers found that the mutant TDP-43 was toxic to neurons and that more of it was found in the cytoplasm. Although the mutant protein

formed inclusion bodies, these did not affect the risk of cell death. However, the amount of cytoplasmic TDP-43 was a strong and independent predictor of neuronal death. Using genetic manipulations, they showed that targeting wild-type TDP-43 to the cytoplasm is sufficient to recreate the toxicity associated with mutant TDP43. On the other hand, the toxic effect of the mutant protein could be blunted by preventing its export from the nucleus. It seems as if the toxicity of the mutation depends on cytoplasmic mislocalization of TDP-43.

"Our results indicate that the mutant protein is mislocalized to the cytoplasm," Finkbeiner said. "Although we don't know the underlying mechanism, the protein seems to become toxic in the cytoplasm and then causes death of the neuron."

Provided by Gladstone Institutes