NEUROSCIENCE

A handle on neurodegenerative disease complexity

Combining experiments and calculations makes it possible to measure the prognostic value of toxic protein species in the cell.

Neurodegenerative diseases are no doubt complex pathologies. It is becoming increasingly clear that several factors contribute to the degeneration and ultimate cell death in Huntington's, Alzheimer's or Parkinson's diseases. Complex problems often require complex solutions. To understand the cellular mechanisms behind these diseases, researchers are turning to systems biology approaches, using both experimental as well as mathematical tools.

Age-related neurodegenerative diseases, such as Huntington's, are often associated with the existence of toxic intracellular proteins. Huntington's is caused by a mutant form of the protein huntingtin (Htt) with an abnormally high number of glutamine amino acids, known as a poly(Q) tract. Mutant Htt forms aggregates inside the neurons of individuals affected with the disease.

It is still not clear though, whether these aggregates are toxic to the cell or whether they represent a way for the cell to cope with the misfolded mutant proteins. Mutant Htt proteins are continuously shifting from one conformation to another, and it is unclear which of the conformational states (or protein species) exist at a given time in the cell and to what extent each of them contributes to the cell's degeneration.

These are not easy questions to address, and as Steven Finkbeiner and his team at the University of California, San Francisco, noted, methods for such investigations have been lacking. To date, a lot of work has focused on looking at aggregation of purified proteins in a test tube. However, "the environment in which proteins fold and misfold inside cells is very different from the one *in vitro*," says Finkbeiner. The group set out to develop new tools and methods that would enable labeling the different protein species of Htt that exist *in situ* and estimating the pathogenic contribution of each of them.

Finkbeiner's team first developed an antibody (3B5H10) to the mutated form of Htt and tested it—along with three other antibodies that recognized different epitopes of the protein—in cultured striatal neurons. The neurons expressed a GFP-tagged form of Htt carrying poly(Q) tracts of different lengths. When the group stained these neurons with each of the antibodies, they found that they generated distinct and reproducible binding patterns. Notably, these binding patterns were predictable. So knowing the length of the poly(Q) expansion, the amount of diffuse Htt (Htt-GFP) and the binding profiles of each of the antibodies, they could use regression analysis to estimate the amount of each epitope inside the cell.

Statistical survival models, such as Cox analysis, are commonly used in clinical research to discover and measure factors that predict a given outcome (for example, the time an indvidual is going to live). Finkbeiner and colleagues used their tools and calculations for a similar purpose: to determine which epitope among many coexisting inside the cell best predicted increased risk of cell death.

The authors tracked thousands of neurons individually over days with an automatic microscope that recorded both the amount of diffuse Htt and the survival time of each neuron. They then correlated the amount of each epitope with the survival time of each cell and applied a modified Cox analysis to determine which epitope out of the four had the best prognostic value.

The 3B5H10 antibody binding pattern predicted with the most accuracy which neurons would degenerate and when one could expect it to happen. The group determined that 3B5H10 binds a specific conformational state of the monomeric (or small oligomeric) form of mutant Htt. "Maybe we need to think about these problems in terms of toxic folds, non-native folds that get exposed in a protein and confer the toxic function," Finkbeiner notes.

It will be very interesting to perform similar studies in other types of neurons that are also affected by the disease and to determine how exactly this toxic protein conformation exerts its deleterious effects in the cell.

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RESEARCH PAPERS

Miller, J. *et al.* Identifying polyglutamine protein species in situ that best predict neurodegeneration. *Nat. Chem. Biol.* **7**, 925–934 (2011).