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Teasing Out the Early Steps of Neurodegeneration

Researchers studying mice that develop a neurodegenerative disorder similar to spinocerebellar ataxia type 1 (SCA1) have pinpointed abnormalities in gene expression that occur long before signs of the disease appear. The researchers believe that their discovery could lay the groundwork for tracing the cascade of malfunctions that ultimately leads to the degeneration of specific groups of neurons in patients with SCA1.

"Our identification of a number of genes that seem important in these early stages (of spinocerebellar ataxia type I) could offer an important foundation for understanding the earliest molecular events that eventually cause the cell to become dysfunctional and die."

— Huda Y. Zoghbi

HHMI investigator Huda Zoghbi at the Baylor College of Medicine and colleagues reported their findings in the February 2000 issue of *Nature Neuroscience*.

In 1993, Zoghbi and her collaborator Harry Orr of the University of Minnesota found that SCA1 is caused by a "genetic stutter" of the three-nucleotide DNA sequence CAG. Genes that contain this stutter have an unusually high number of CAG repeats, which in turn causes them to produce proteins with an abnormally long glutamine tract. A normal *SCA1* gene has about 30 CAG repeats, which are interrupted by another triplet repeat sequence. People who have SCA1, however, carry a gene with 40 to a hundred uninterrupted CAG repeats.

SCA1 is one of eight neurodegenerative diseases the most well known of which is Huntington's disease that is caused by this type of mutation. Somehow, the "polyglutamine" proteins cause severe degeneration in specific groups of neurons, which vary from one disease to the next. Patients with

SCA1 suffer the worst damage in their cerebellar Purkinje cells, and consequently lose their balance and motor coordination. Loss of muscle control worsens steadily until patients are no longer able to eat or to breathe.

In 1998, Zoghbi and her colleagues showed that the abnormal SCA1 protein, ataxin-1, accumulates in the cell nucleus where it presumably impairs normal functions.

"After those studies, we were left with two major questions," said Zoghbi. "We wanted to understand how the accumulation of ataxin-1 affected the normal function of the cell's nucleus. And, we wanted to understand why Purkinje cells were most severely affected, even though ataxin-1 is present in all brain cells."

To answer these questions, the researchers used a mouse model of SCA1 that mimicked the human disease. Like humans with SCA1, the mice were born with a *SCA1* gene containing an excessive number of glutamine repeats and suffered Purkinje cell degeneration.

Using a technique known as "PCR-based cDNA subtraction," which allowed them to compare gene expression in normal and SCA1 mice, Zoghbi and colleagues were able to pinpoint other genes whose expression pattern was altered by the abnormal *SCA1* gene. "We found six neuronal genes, all highly abundant in Purkinje cells, that were downregulated at a surprisingly early stage in the disease," said Zoghbi. "In fact, the earliest gene, called *PCCMT*, was downregulated only one day after the *SCA1* gene turns on. Others were downregulated a few days or weeks afterward."

Also surprising, said Zoghbi, was that several of the downregulated genes produced proteins that helped regulate calcium levels in neurons. "This finding suggests that calcium physiology and homeostasis is altered in the affected Purkinje cells," said Zoghbi. Calcium is crucial to a neuron's ability to send and receive electrochemical signals.

The researchers found that a mouse homolog of the human gene called α *I-ACT*, which is perturbed in patients with Alzheimer's and Huntington's diseases, was also upregulated in their transgenic mice. According to Zoghbi, the discovery that this gene is affected suggests a possible commonality among the diseases in the reactions of brain cells to earlier malfunctions.

In comparing the gene expression profiles from their mouse model to gene expression in human tissue from SCA1 patients, Zoghbi and her colleagues found similarities in the patterns of expression of the affected genes.

"In humans, these neurodegenerative diseases do not show up until later in life, so the changes in cells must be very subtle," said Zoghbi. "Thus, our identification of a number of genes that seem important in these early stages could offer an important foundation for understanding the earliest molecular events that eventually cause the cell to become dysfunctional and die."

Future experiments, said Zoghbi, will concentrate on understanding how the mutant ataxin-1 protein interacts with genes in the nucleus. In particular, said Zoghbi, she and her colleagues would like to understand the function of *PCCMT*, the first gene to be downregulated, since it might be a key to later malfunctions in the cell.

Zoghbi emphasizes that the scientists' basic discoveries will not likely lead to direct clinical applications.

"Nevertheless, if we could understand the early perturbations in calcium homeostasis in these Purkinje cells, and if these could be pharmacologically altered, these basic findings might ultimately aid treatment of this disease," she said.