Researchers implicate wayward DNA-repair enzyme in Friedreich's ataxia

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Scripps Research Institute scientists have taken a step closer to understanding the cause of Friedreich's ataxia, a debilitating neurological condition that affects tens of thousands of people worldwide, and so far has no cure.

Researchers already know that the disease arises from the silencing of the gene FXN, due to an abnormally expanded stretch of DNA within the gene. The length of this "triplet repeat expansion" determines the degree of gene silencing, and thus the severity of the disease. This length also tends to change from carrier parents to patient offspring. But the cause of this genetic instability hasn't been clear.

After creating a stem cell model of the disease from patients' skin cells, the Scripps Research scientists found strong evidence for the involvement of a DNA repair enzyme in the FXN triplet repeat expansion. The finding suggests that the expansion is driven by the enzyme's misguided attempts to repair what it recognizes as DNA damage.

"We've known that in other triplet repeat expansion diseases, such as Huntington's disease, DNA repair enzymes are somehow involved in generating the expansion," said Joel M. Gottesfeld, PhD, a professor at The Scripps Research Institute, and the study's senior author. "But this is the first time this has been demonstrated for Friedreich's ataxia. It's also the first time that anyone has created a model system to study the triplet repeat expansion phenomenon in patients' own genes."

The study was published in the November 5, 2010 issue of the journal *Cell Stem Cell*.

The Black Flag

Friedreich's ataxia is the most common form of ataxia, a condition in which coordinated muscle movements are lost. Its symptoms first appear in childhood or early adulthood, and often go on to include speech and vision problems, and in later life serious heart problems. Most patients are eventually confined to a wheelchair, and few live past age 50.

The nerve degeneration that causes the disease arises from the absence of the protein frataxin, normally produced by the FXN gene. Frataxin plays a role in protecting tiny cellular energy-factories known as mitochondria, and without it, cells are more likely to die an early death. The cells that are most vulnerable to this process are muscle-related sensory neurons whose degeneration leads to the first symptoms of the disease.

Gottesfeld's laboratory has been deeply involved in Friedreich's ataxia research, and has already performed work on the initial development of a drug candidate now about to be tested in clinical trials by Repligen Corporation. But in the present study, Gottesfeld and his colleagues wanted to learn more about the cause of the ever-shifting DNA expansion that shuts down the FXN gene.

In a normal, working copy of the gene, there is a section containing a pattern of three DNA nucleotides – guanine, adenine, adenine (GAA) – repeated up to about 20 times. In Friedreich's ataxia patients, this GAA repeat section is vastly expanded and typically contains hundreds of repeats. Somehow this expansion flags the machinery of the cell nucleus to silence the gene, by packing its DNA into a more tightly twisted form, known as heterochromatin.

Getting at the cause of the expansion hasn't been easy. "We haven't had human cellular models that faithfully reproduce this feature of the disease," said Gottesfeld.

New Clues from a New Model

In the study, members of the Gottesfeld lab, led by first author Sherman Ku, a graduate student in the Scripps Research Kellogg School of Science and Technology, created a cellular model that did reproduce this feature. Ku started by selecting Friedreich's ataxia patients' skin cells from a cell library. Using a technique known as transcription factor reprogramming, first described by the Japanese stem-cell pioneer Shinya Yamanaka and his colleagues in 2007, Ku "rebooted" the skin cells' genetic machinery to turn them into stem cells.

In the stem cells, as in the skin cells, FXN expression stayed very low. But the stem cells also appeared to model the generation-to-generation repeat expansion seen in human patients. "In the transition from skin cells to stem cells, and then as the stem cells grew in culture and divided to produce their own daughter stem cells, the triplet repeat expansions within FXN became longer," said Ku.

Ku ran a number of further experiments in the cells to find out what was causing the expansion. In one, he found elevated expression levels of the gene MSH2, whose product is a known DNA repair enzyme. In another experiment, he found evidence that the enzyme had bound to the FXN gene close to the triplet repeats.

"In a final experiment," said Ku, "we silenced the expression of MSH2 in the cells, using a technique known as RNA interference, and found that the triplet repeat expansion was partially arrested when the cells divided."

The study lends support to a general hypothesis for triplet repeat expansion diseases. "The idea is that these repeats can form unusual DNA structures, which enzymes such as MSH2 incorrectly identify as mismatched DNA," said Gottesfeld. "During the course of their attempted repair they cause extra repeats to be synthesized and inserted into the gene."

Unfortunately, he adds, developing a drug that stops MSH2 isn't an option, because people could become even less healthy if they lose some of their DNA repair capability. Mutations in the MSH2 gene are already known to cause an inherited form of colorectal cancer.

"With the new stem cell model, however, we hope eventually to put together a much more detailed picture of this process of repeat expansion and gene silencing, and that should put us in a good position to devise optimal therapies," Gottesfeld said.

More information: "Friedreich's ataxia induced pluripotent stem cells model intergenerational GAA*TTC triplet repeat instability," *Cell Stem Cell*.

Provided by The Scripps Research Institute