



LOU GEHRIG'S DISEASE

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## DISCOVERIES SHOULD AID RESEARCH INTO CAUSE OF ALS

Two teams of researchers at Northwestern University have found a novel pathological hallmark of the neurodegenerative disease amyotrophic lateral sclerosis (ALS) at the molecular level. The neurologists and biochemists show how and why the mutated superoxide dismutase (SOD1) protein, which is associated with a familial form of ALS, becomes vulnerable and prone to aggregation and also provide evidence linking disease onset with the formation of intermolecular aggregates.

The findings, which have implications for new therapeutics for the devastating disease, were published online this week in two related papers by the Proceedings of the National Academy of Sciences (PNAS).

ALS is a progressive paralytic disorder caused by degeneration of motor neurons in the brain and spinal cord. The cause and development (pathogenesis) of the fatal disease are not known, and there is no effective treatment. Fifteen years ago, an international consortium led by Teepu Siddique, M.D., Les Turner ALS Foundation/Herbert C. Wenske Foundation Professor at Northwestern's Feinberg School of Medicine, mapped the first ALS gene to chromosome 21. Subsequently, they found that mutations in the SOD1 gene are responsible for 20 percent of familial (inherited) ALS cases. Siddique and his colleagues also made the first ALS transgenic mouse models.

Although more than 100 types of a single mutation in the SOD1 gene have been identified and multiple lines of the mouse models developed, a key question remains to be answered: How does the genetic mutation alter this incredibly stable protein to make it so toxic that it kills motor neurons and causes neurodegenerative disease?

The presence of aggregated proteins is common to many neurodegenerative disorders, including ALS and Alzheimer's, Parkinson's and prion diseases, but the relevance of these aggregates to the diseases is not well understood. In ALS patients with SOD1 mutations and mouse models overexpressing mutant SOD1, SOD1-positive aggregates were identified in neurons. Researchers do not know if these aggregates are

causative, harmless or even beneficial to ALS. Furthermore, the fundamental molecular mechanism by which the SOD1 mutants form aggregates is not clear.

Six years ago Siddique and Han-Xiang Deng, M.D., associate professor of neurology at the Feinberg School, started to develop and analyze various SOD1 transgenic mouse models and found, as they report in the first of the two PNAS papers, that aggregated and insoluble SOD1 is the pathogenic form that causes disease. The aggregation takes place in mitochondria, the powerhouse of the cell, which becomes damaged.

“We also have discovered a mechanism whereby ‘normal’ molecules of SOD1 are recruited in the presence of mutant SOD1 proteins to participate in the pathogenesis of ALS by forming intermolecular disulfide bonds,” said Siddique. “This phenomenon is in some ways akin to the recruitment noted in prion disorders and provides molecular sites for therapeutic intervention.” This molecular mechanism also may help explain other types of ALS in which no mutations have been detected in SOD1.

The normal form of SOD1 is a molecule composed of two identical parts, each with an amino acid chain, a copper ion, a zinc ion and an intramolecular disulfide linkage -- a bond within an SOD1 molecule that stabilizes the structure. Intermolecular disulfide bonds, or cross-links, are incorrect bonds that form between, not within, SOD1 molecules.

“A year ago we demonstrated that ALS mutations have the greatest effect on the most immature form of the SOD1 protein, causing it to misfold and form incorrect disulfide bonds that facilitate protein aggregation,” said Thomas V. O’Halloran, professor of chemistry. “Those were test tube experiments, but we really wanted to know if we would find the same process in a physiological environment.”

To investigate their hypothesis further, O’Halloran and Yoshiaki Furukawa, formerly a post-doctoral fellow in O’Halloran’s lab, teamed up with Siddique and Deng. As reported in the second PNAS paper, they show that increased oxidative stress leads to the mutant SOD1 protein forming incorrect disulfide bonds early in its life. The researchers isolated and examined aggregates from the spinal cord of several ALS-model mice. In the diseased mice the scientists found intermolecular disulfide bonds that cross-linked SOD1 molecules together, resulting in the formation of insoluble aggregates. The SOD1 aggregates were specific to the spinal cord, the part of the body most damaged in ALS. Tissues unaffected by the disease, such as the brain and liver, had no aggregates.

Oxidation and protein aggregation have been suspected to play an important role in the pathogenesis of neurodegenerative disorders as well as in the normal aging process. However, the relationship between protein oxidation, protein aggregation and neurodegeneration remains unclear. The oxidative intermolecular disulfide cross-linking paradigm established by the Northwestern researchers provides direct links between protein oxidation, protein aggregation and neurodegeneration in SOD1-mediated ALS. This mechanism may play an important role not only in SOD1-mediated ALS but also in some other neurodegenerative disorders.

“For some time researchers have been thinking that copper, which causes the protein to be bluish-green in color, was the bad guy in this disease,” said O’Halloran. “But the data has been building up to say that something else may be responsible for the toxicity. Our results suggest that the status of the disulfide bond, a long overlooked part of the SOD1 protein, plays a pivotal role. The ALS mutations appear to predispose SOD1 to form incorrect disulfide bonds that lead to aggregation of the protein and perhaps initiation of the disease. If these ideas are borne out, our next step is to look for therapeutic approaches that could prevent formation of the disulfide cross-linked aggregates.”

In addition to Siddique, Deng (lead author), O’Halloran and Furukawa, other authors on the paper titled “Conversion to the ALS phenotype is associated with insoluble aggregates of intermolecular linked mutant and wild type SOD1 in mitochondria” are Yong Shi, Hong Zhai, Ronggen Fu, Erdong Liu, George Gorrie, Mohammad S. Khan, Wu-Yen Hung, Eileen H. Bigio, Thomas Lukas and Mauro C. Dal Canto, all from the Feinberg School of Medicine. The paper can be viewed at <http://www.pnas.org/cgi/doi/10.1073/pnas.0602048103>.

In addition to O’Halloran, Furukawa (lead author), Siddique and Deng, the other author on the PNAS paper titled “Disulfide cross-linked protein represents a significant fraction of ALS-associated SOD1 aggregates in spinal cords of model mice” is Ronggen Fu, research technologist at the Feinberg School. The paper can be viewed at <http://www.pnas.org/cgi/doi/10.1073/pnas.0602046103>.

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