The role of metal ions in amyotrophic lateral sclerosis

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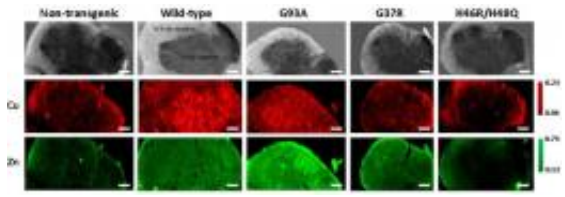

Infrared and x-ray fluorescence microscopy (XFM) images of half a spinal cord cross section from a normal mouse (non-transgenic), a healthy mouse expressing normal SOD (wild-type), and diseased ALS mice with SOD mutations (G93A, G37R, and H46R/H48Q). The top row contains infrared data showing the lipid-rich white matter around the protein-rich gray matter. The second and third rows show the copper and zinc content from x-ray fluorescence microprobe X27A. Copper was decreased in gray matter of the H46R/H48Q mutations, which does not bind copper. All of the spinal cords from the sick mice contain more zinc in the white matter compared to the healthy mice. The white scale bar represents 0.1 mm, and the XFM scale bars are in concentration units of mM.

(PhysOrg.com) -- Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a progressive neurodegenerative disease that affects motor neurons in the spinal cord, leading to muscle weakness, paralysis, and death within two to five years. With a lifetime risk of 1 in 2,000, ALS is the most common motor neuron disease.

Approximately 90 percent of all ALS cases are sporadic in nature, with no known cause. However, the remaining 10 percent are inherited. Of

these genetic cases, 20 percent are linked to mutations in the metal-containing protein superoxide dismutase (SOD1), which is an important antioxidant enzyme that requires zinc for structural stability and copper for its detoxifying function. Interestingly, over 145 different mutations in SOD1 have been identified in patients with ALS.

In a study recently published in the Journal of Biological Chemistry, our team at BNL and collaborators from UCLA, the University of Florida, and Stony Brook University used mouse models of ALS to understand how metal ions and SOD1 mutations play a role in the disease process. These mice were genetically predisposed to develop ALS-like disease by over-producing human forms of mutated SOD1. Similar to human patients, they develop aggregates of SOD1 in the spinal cord and undergo progressive paralysis. To explore the role of metal ions in aggregation and disease, we analyzed copper and zinc in the spinal cord tissue in two different ways. First, we determined whether the SOD1 protein molecules were fully "metallated," which makes the protein functional. Second, we examined the overall distributions of copper and zinc in the spinal cord.

For the first part of the study, SOD1 was gently extracted from the spinal cord and separated into soluble (non-aggregated) and insoluble (aggregated) fractions, which were analyzed for metal content. The results showed that the aggregates did not contain the necessary metal ions for the proteins to be functional. In contrast, the soluble SOD1 contained the expected amount of copper and zinc needed for the protein to function. Since SOD1 is highly stable once it is fully metallated, these findings support the hypothesis that the aggregates of mutated SOD1 are derived from immature protein before it has a chance to acquire the necessary copper and zinc ions.

In the second part of the study, NSLS beamline X27A was used to image the distribution of copper and zinc in cross-sections of the spinal cords.

For those mutations where SOD1 was able to bind copper ions, results showed that copper was redistributed to regions with high SOD1, leaving other regions of tissue copper-deficient. The zinc distribution followed a different trend, where a high concentration of zinc was found in the spinal cord's "white matter" for all mutations, regardless of the mutation's ability to bind metal ions. Since the white matter is the region of the spinal cord where nerve transmission occurs, high zinc content may indicate short-circuiting or death of neurons.

As a result of these studies, we have new information about the progression of ALS. For example, it is likely the aggregates in ALS arise from newly formed SOD1, prior to metallation. Once copper and zinc bind to SOD1, it becomes very stable and is no longer susceptible to aggregation. Thus, one treatment approach to ALS may involve methods for metallating SOD1 prior to aggregate formation. In addition, the disease process induces a redistribution of metal ions in the spinal cord, further compromising the tissue metabolism. Interestingly, the change in zinc content may provide a diagnostic marker of the disease process, and future studies at earlier stages of the disease will investigate this possibility.

More information: H.L. Lelie, et al., "Copper and Zinc Metallation Status of Copper-Zinc Superoxide Dismutase from Amyotrophic Lateral Sclerosis Transgenic Mice," *Journal of Biological Chemistry*, 286, 2795 (2011).

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