Chemist devises new method to quantify protein changes

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A scientist from the Florida campus of The Scripps Research Institute has devised a new method of analyzing and quantifying changes in proteins that result from a common chemical process. The new findings could provide new insights into the effects of a highly destructive form of stress on proteins in various disease models, particularly cancer.

The study, published January 5, 2011, in the online Early View of the journal Angewandte Chemie, was designated by the journal as a "very important paper," a distinction bestowed on less than five percent of its publications.

"This new technique allows us to home in on which proteins are modified to a significant extent during periods of stress and how that changes during disease progression," said Kate Carroll, an associate professor in the Scripps Research Department of Chemistry who conducted the study with Young Ho Seo, a research fellow at The University of Michigan. "It gives us the chance to look more closely at targets for possible therapeutic intervention. From a practical standpoint, the technique is simple and will be accessible to biologists and chemists alike."

The new technique focuses on the process of cysteine S-hydroxylation, which plays a significant role in a number of events related to physiology in both health and disease, including the regulation of signaling proteins in various disease states.

The ability of the new technique to focus on signaling pathways, particularly in diseases such as cancer, is critical.

"Chronic disease states such as cancer can involve the modification of signaling proteins through S-hydroxylation, but other housekeeping proteins may also be targets," she said. "Key to distinguishing which of these proteins may be involved in pathogenesis is the ability to measure the amount of S-hydroxylation at specific sites within a protein. Now you'll be able to tell. This should help accelerate target identification in these disease-related signaling pathways and allow us to focus on proteins that are important to the process."

During periods of cellular stress, caused by factors such as exposure to UV radiation or many disease states, the level of highly reactive oxygen-containing molecules can increase, resulting in inappropriate modification of proteins and cell damage.

One oxidant, hydrogen peroxide, functions as a messenger that can activate cell proliferation through oxidation of cysteine residues in signaling proteins, producing sulfenic acid (i.e., S-hydroxylation); cysteine is an amino acid is synthesized in the body.

Extending the Gains of an Earlier Study

In a 2009 study, Carroll found that sulfenic acid served as an early warning biomarker of the reaction between hydrogen peroxide and cysteine. Carroll tagged the miniscule reaction target with a fluorescent dye antibody. With it, Carroll was able to read the levels of sulfenic acid levels in various cell lines, including breast cancer cells.

The new technique takes those findings several steps further by allowing scientists not only to quantify the modifications to various proteins, but also to monitor these changes at the level of individual cysteines within a

single protein.

Carroll used a class of reagents called isotope-coded dimedone and iododimedone, which traps and tags sulfenic acids, allowing the cysteine sites and modified proteins to be easily identified. These probes, which are highly selective for sulfenic acid, allow the S-hydroxylation process to be monitored at the exact site of the modification.

The tagged proteins can be then be analyzed by mass spectrometry, a standard technology used to determine the precise make-up of proteins and other molecules.

"This technique should be widely accessible to the scientific community because it's so simple," Carroll said. "It should allow researchers to identify proteins with altered S-hydroxylation profiles whose function may lend insight into events in disease progression and have utility as potential markers for disease detection."

More information: For more information on the paper, see onlinelibrary.wiley.com/doi/10 … e.201007175/abstract

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