New technique reveals cross-talk between two essential cellular processes

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Scientists at The Scripps Research Institute have simultaneously mapped two of the most important types of protein-modification in cells, revealing their extensive cooperation during an essential cellular process.

Phosphorylation, the attachment of a phosphate group to a protein, and proteolysis, the cleavage of a protein, had almost always been studied independently. The new research combines techniques for mapping these events across all proteins in a cell population to show how they work together to execute the cellular "auto-destruct program" known as apoptosis.

The specific findings on apoptosis may lead to the development of new cancer diagnostics and drugs, since cancer treatments often aim to induce apoptosis in malignant cells. Even more importantly, the study marks the development of a basic new tool of "proteomics"—the large-scale study of proteins—that should provide useful insights into many cellular processes.

"Detecting the cross-talk between protein regulation pathways has long been a challenge, and so with this new technique we can start to do analyses that were difficult or impossible before," said Benjamin F. Cravatt, professor and chair of the Department of Chemical Physiology at Scripps Research, and member of Scripps Research's Skaggs Institute for Chemical Biology. Cravatt was the senior investigator for the study, published in the July 20, 2012 edition of the journal *Cell*.

Need for a Global Approach

Phosphorylation and proteolysis are among the most important mechanisms of protein modification in cells. They are mediated by enzymes, and occur after a protein has been translated from genetic material and folded. Some proteolysis and phosphorylation events serve to activate a protein so that it can take part in a purposeful cellular process; others have the effect of deactivating a protein.

Previous studies of phosphorylation and proteolysis had suggested that the two mechanisms sometimes work in tandem, especially during apoptosis. But those studies had been focused on individual apoptosis-driving enzymes and their biochemical partners, rather than on the "global" apoptosis process within cells.

"In this study, we wanted to develop a global method to let us see all the signs of cross-talk between proteolysis and phosphorylation during apoptosis," said Melissa M. Dix, a research associate in the Cravatt laboratory. Dix was a lead author of the paper, along with then-graduate student Gabriel M. Simon, who is now a postdoctoral researcher at Washington University, St. Louis.

New Insights

Dix and Simon built on an earlier proteolysis-mapping method that they had described in Cell in 2008. Known as PROTOMAP, it can be used to generate a detailed picture of the protein cleavage events in cells during a process of interest. For the new study, the researchers added a technique for detecting phosphorylation events, plus another recently-developed proteomics technology, SILAC, which enables researchers to distinguish, within a given sample, copies of proteins that have come from different cell populations. The researchers then applied the

combined techniques to populations of control cells and apoptotic cells, in order to find the proteolysis and phosphorylation events that happened only during apoptosis.

They detected more than 700 apoptosis-specific proteolysis events—mostly mediated by apoptosis-driving enzymes known as caspases—including many that had not been reported before. The new mapping also revealed for the first time an extensive, apoptosis-specific network of phosphorylation events, many of which were clearly connected to proteolysis events. "Just looking at the map of phosphorylation events, we could see that they were unusually common around sites of known caspase cleavage," said Dix.

Previous studies had hinted that the phosphorylation of a protein near one of its caspase cleavage sites would always tend to block that cleavage. The new evidence suggested otherwise. "We could see that these apoptosis-specific phosphorylations sometimes persisted on caspase-cleaved fragments," said Dix.

Dix and Simon showed that these phosphorylations in some cases had enabled the caspase cleavage events; in others, cleavage events had enabled the phosphorylations. Similarly, they confirmed that some of the kinase enzymes that phosphorylate proteins during apoptosis can't do their jobs until they are cleavage-activated by caspases. "We've tended to study proteolysis and phosphorylation separately, but it's clear that they're intimately associated and need to be looked at as such," Dix said.

Potential Applications in Cancer Treatment

The Cravatt laboratory is now applying the techniques developed in this study to other analyses, starting with studies of apoptosis in a variety of cell types. The cells they used in the just-published study, Jurkat T-cells, are often used to investigate apoptosis because they can be easily

induced to undergo the process. "But each cell type has its own set of working proteins, which will give it a distinct signature when it undergoes apoptosis," Cravatt said.

Techniques to detect apoptosis in specific cell types would be useful in cancer diagnostics and therapy. Tumor cells typically have evolved resistance to apoptosis, whereas chemotherapies often kill tumor cells by overcoming that resistance. Dix, Cravatt, and their colleagues are now trying to determine whether certain phosphorylated protein fragments can be used as highly specific "biomarkers" of apoptosis in cancer cells, detectable in a simple blood test. "Such a test would tell you whether or not your cancer drug is working," Cravatt said.

The wealth of data from these apoptosis studies may also help researchers devise new apoptosis-inducing cancer drugs, he notes—and apoptosis is merely one cellular process that the new mapping technique can be used to illuminate.

More information: "Functional interplay between caspase cleavage and phosphorylation sculpts the apoptotic proteome," *Cell*.

Provided by Scripps Research Institute

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