Study dusts sugar coating off little-known regulation in cells

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In Alzheimer's disease, brain neurons become clogged with tangled proteins. Scientists suspect these tangles arise partly due to malfunctions in a little-known regulatory system within cells. Now, researchers have dramatically increased what they know about this particular regulatory system in mice. Such information will help scientists better understand Alzheimer's and other diseases in humans and could eventually provide new targets for therapies.

In a study released online in the Proceedings of the National Academy of Sciences Early Edition this week, the team at least doubled the number of proteins found to be subject to a type of regulation based on a sugar known as O-GlcNAc (oh-GLIK-nak). The O-GlcNAc system likely adds another layer of control to the proteins that serve as a brain cell's widgets and gears -- control that might be muddled in Alzheimer's brains known to have problems in sugar metabolism.

"We found many novel proteins providing insights into new aspects of cell biology," said analytical biochemist Feng Yang of the Department of Energy's Pacific Northwest National Laboratory and lead author on the study. "We think O-GlcNAc is fine-tuning cellular processes."

In addition to finding hundreds of proteins modified by O-GlcNAc, the team found that almost all the O-GlcNAc proteins were also subject to the most common form of protein regulation, which uses small phosphate molecules to turn proteins on and off. This suggests a larger coordination between the two regulatory systems.

"These results show there's a level of complexity about how biology operates that we've been largely blind to," said PNNL's Richard D. Smith, who leads the proteomics team at PNNL. Proteomics researchers try to understand how a cell functions based on the numbers and types of its proteins at work, which are collectively known as the proteome (PRO-tee-ohm).

"Back during the Human Genome Project, we asked, how could so few genes produce the complexity of an organism or even a single cell, and how could minor variations in our DNA explain the diversity we see all around us? Clearly the proteome is the answer," said Smith.

Sugar Switch

Proteins are the tools, gears and gadgets that run a cell. Regulatory systems within cells turn proteins on and off by attaching or detaching small molecules to the proteins, like a switch. The most common switch involve adding or removing phosphates, and biologists have known for a long time that these switches can run amiss in cancer and other diseases. Drugs affect players in the phosphate regulatory system to try to fix the errors.

A couple decades ago, researchers found that O-GlcNAc, a kind of sugar, could also work like a switch, turning proteins on or off. Scientists found proteins decorated by O-GlcNAc, as well as other proteins that attach or remove the sugar -- all essential parts to the system.

But they had trouble finding enough O-GlcNAc proteins to get the whole story. Few proteins bore the small sugar, and those that did tended to lose the accessory while being manhandled in the lab. Researchers could make up for some of these problems by starting with more tissue or cultured cells, but they knew if they wanted to look for these modifications in real-life scenarios such as clinical samples, they would

need to be able to find the sugar with a small amount of starting material.

To overcome these difficulties, Smith, Yang and their colleagues at PNNL and four research institutions combined their expertise in the O-GlcNAc system with instruments developed at EMSL, DOE's Environmental Molecular Sciences Laboratory on the PNNL campus. First they improved how they purified protein from mouse brain tissue to reinforce the sugar attached to proteins. Then they used instruments that exceled at detecting rare proteins in small samples.

In addition, they looked for the sugar-dotted proteins in mouse brain samples from engineered animals that had a mouse version of Alzheimer's. These mice make too much of three key proteins implicated in Alzheimer's disease in people, including the Tau protein, which forms the hallmark tangles in brain neurons.

Pack o' Proteins

To test how well their methods found O-GlcNAc proteins, the PNNL-led team started with tissue from either healthy or diseased mouse brain tissue. From the healthy tissue, the team found 274 different proteins marked with O-GlcNAc. Many of them sported more than one sugar molecule, because the team found a total of 458 attachment sites on those 274 proteins -- triple the number of sites found in any previous study. The large number of sites allowed the team to identify similarities between O-GlcNAc sites, as well as O-GlcNAc sites on previously unexplored proteins.

Of the 274 O-GlcNAc proteins, 106 had already been identified in other studies. That left 168 newly-identified proteins. Based on what the proteins looked like, the team classified most of them as likely being involved in cell signaling, regulating how genes are expressed, or, again, in cell scaffolding.

The O-GlcNAc-dabbed proteins held a variety of jobs, including forming part of a cell's scaffolding, or in nerve growth or in other nerve-related occupations such as learning and memory.

The PNNL-led team then looked at the proteins found in the Alzheimer's-like mouse brain. They found about a third fewer O-GlcNAc-marked proteins. That result also supports earlier work that suggested there is damaged O-GlcNAc regulation in Alzheimer's brains in people.

Fraternizing Phosphates and Other Biology

One of the more exciting things the researchers found had to do with the most common regulatory system in cells, the phosphate system. More than 98 percent of the O-GlcNAc proteins also had sites that would accept a phosphate, suggesting those proteins are also under the control of that system.

And about a quarter of the O-GlcNAc sites were close enough to the phosphate sites to interfere with that switch, suggesting cross-talk between the two types of regulation. A phosphate is smaller than O-GlcNAc and has a strong negative electrical charge. The sugar is neutral but bulkier. Those characteristics could have different effects on the structure of the protein and greatly increases the range of possible biological effects due to the complexity of the combined switching systems.

Lastly, until this study, most of the proteins known to be under O-GlcNAc control largely live their lives within the cells. But the PNNL-led team found a half-dozen proteins that had to be controlled by O-GlcNAc outside a cell, based on where their O-GlcNAc site fell on the body of the protein.

Now, the team is planning to measure both regulatory systems in concert.

"It's revealing to see how many proteins are modified. If we're going to understand biological systems, we need to understand the interplay of the different types of modifications," said Smith.

More information: Joshua F. Alfaro, Cheng-Xin Gong, Matthew E. Monroe, Joshua T. Aldrich, Therese R.W. Clauss, Samuel O. Purvine, Zihao Wang, David G. Camp II, Jeffrey Shabanowitz, Pamela Stanley, Gerald W. Hart, Donald F. Hunt, Feng Yang, and Richard D. Smith, 2012. Tandem Mass Spectrometry identifies many mouse brain O-GlcNAcylated proteins including targets of an EGF domain-specific OGT, *Proc Natl Acad Sci* Early Edition online the week of April 16, DOI 10.1073/pnas.1200425109

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