Sugary secrets of a cancer-related protein

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The proteins in human cells are extensively decorated with different types of sugars, a phenomenon called glycosylation. These modifications greatly increase the diversity of protein structure and function, affecting how proteins fold, how they behave, and where they go in cells. New research that will be published in the *Journal of Biological Chemistry* on Sept. 22 demonstrates that a rare type of glycosylation profoundly affects the function of a protein important for human development and cancer progression.

Protein glycosylation is either called N-linked or O-linked, depending on whether the sugar is attached to nitrogen- or oxygen-containing sites, respectively. O-linked modifications typically involve the sugar N-acetylgalactosamine being attached to the amino acids serine or threonine, called "mucin-type" glycosylaton because they are commonly found in proteins in mucus membranes; together with N-linked sugars, these "canonical" modifications modify thousands of different types of proteins.

For over 20 years, Robert Haltiwanger's research group, now at the University of Georgia, has studied much rarer type of O-linked modifications: attachment of the sugars glucose or fucose to serine or threonine, a modification that affects just a few hundred different types of proteins. One of these proteins is Notch, a signaling receptor that is essential for cell development and differentiation and is dysregulated in cancers such as leukemia, breast cancer, and prostate cancer.

"The fact that we found these sugars on Notch was intriguing because

Notch is a very important molecule," Haltiwanger said. "So we've been curious about how these sugars affect [Notch's] stability and activity."

The enzymes responsible for modifying Notch with glucose and fucose are called POFUT1 and POGLUT1. Haltiwanger's team, led by Hideyuki Takeuchi, wanted to know exactly why POFUT1 and POGLUT1 were attaching glucose and fucose to Notch in cells.

If you genetically engineer a fly or mouse without POFUT1 or POGLUT1, Haltiwanger said, "you get a dead fly or a dead mouse. You completely disrupt the Notch pathway; Notch is not functional if you don't add those sugars. There's been a lot of work over the years on: Why is that? What is [the sugar] doing?"

Haltiwanger's new work shows that the fucose and glucose modifications serve as quality-control markers that allow Notch to be transported to its final destination in the cell membrane. When the researchers knocked out POFUT1 or POGLUT1 in cell cultures using CRISPR/Cas technology, cells displayed much less Notch on the cell surface. When both enzymes were knocked out, Notch was almost completely absent. Using additional biochemical methods, the researchers found that POFUT1 and POGLUT1 attached glucose and fucose to portions of Notch only after they fold in a specific way.

"It's like a stamp of approval," Haltiwanger said. "This part's folded? Boom, you put a fucose on it. And somehow that tells the cell: Don't mess with this anymore. Leave it alone. If you don't add the sugar, [the Notch proteins] get stuck inside the endoplasmic reticulum, get degraded, and don't get secreted."

Knowing that these sugars are essential for Notch activity makes the enzymes that control them, POFUT1 and POGLUT1, potential targets for cancer treatments. Depending on whether Notch is overactive or

insufficiently active in a particular cancer, manipulating the sugars that are added to Notch could help correct the dysregulation. Haltiwanger's team is currently working on finding chemical compounds that would inhibit POFUT1 and POGLUT1, thus stopping Notch from embedding in the cell membrane and carrying out its signaling functions. They're also attempting to unravel the details of how the glucose and fucose modifications work together to fine-tune Notch activity.

"That'll keep us busy," Haltiwanger said.

More information: Hideyuki Takeuchi et al, O-Glycosylation modulates the stability of epidermal growth factor-like repeats and thereby regulates Notch trafficking, *Journal of Biological Chemistry* (2017). DOI: 10.1074/jbc.M117.800102

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