New tools developed to unveil mystery of the 'glycome'

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Scientists at The Scripps Research Institute have developed chemical compounds that can make key modifications to common sugar molecules ("glycans"), which are found on the surface of all cells in our body. The new study presents powerful new tools for studying these molecules' function, for example in cell signaling and immunity, and for investigating new treatments for chronic inflammation, autoimmune diseases, cancer metastasis, and related conditions.

The new study, which appears online in *Nature* Chemical Biology on June 10, 2012, describes compounds that selectively block the attachment to the cell of two types of sugar building blocks, sialic acid and fucose, which are found at the tips of cell surface glycans and can be critical to cell function.

"We've developed the first compounds that can easily get into cells and selectively shut down the enzymes that decorate glycans with sialic acid or fucose," said Scripps Research Professor James C. Paulson, the senior author of the new report.

One of the Least Understood Domains of Biology

The "glycome"—the full set of sugar molecules in living things and even viruses—has been one of the least understood domains of biology. While the glycome encodes key information that regulates things such as cell trafficking events and cell signaling, this information has been relatively

difficult to "decode." Unlike proteins, which are relatively straightforward translations of genetic information, functional sugars have no clear counterparts or "templates" in the genome. Their building blocks are simple, diet-derived sugar molecules, and their builders are a set of about 250 enzymes known broadly as glycosyltransferases. Characterizing these enzymes is essential to understanding the glycome. But one of the most basic tools of enzyme characterization—a specific enzyme inhibitor that can work in cell cultures and in lab animals—has been lacking for most glycosyltransferase families.

Three years ago, Cory Rillahan, a PhD candidate working in Paulson's laboratory, set out to find compounds that can specifically inhibit two important families of glycosyltransferases: the fucosyltransferases, which attach fucose groups, and the sialyltransferases, which attach sialic acids.

"They tend to be the most biologically relevant, because they attach these sugar units at the very tips of the glycan chains, which is where proteins on other cells bind to them," said Rillahan.

Rillahan began a quest by developing a screening technique that could be used to sift rapidly through chemical compound libraries to find strong inhibitors of these two enzyme families. This high-throughput screening technique was described last year in the journal Angewandte Chemie. But while Rillahan waited to get access to a larger compound library, he read of a more focused, rational-design strategy that Canadian researchers had used to devise inhibitors of a different glycosyltransferase.

Using 'Imposter' Molecules

Rillahan quickly adapted this broad strategy against sialyl- and fucosyltransferases in work described in the new study.

Normally an enzyme such as a fucosyltransferase grabs its payload—fucose, in this case—from a larger donor molecule, then attaches the small sugar to a glycan structure. Rillahan created fucose analogs, "impostor" molecules that can be readily taken up by this process, but then jam it. When one of these fucose analogs gets into a cell, it is processed into a donor molecule and grabbed by a fucosyltransferase—but can't be attached to a glycan. Rillahan also designed sialic acid analogs that have the same spoofing effects against sialyltransferases.

These analogs act as traditional enzyme inhibitors in the sense that they bind to their enzyme targets and thereby block the enzymes from performing their normal function. But Rillahan found that his analogs have a second effect on their targeted enzyme pathways. They lead to an overabundance of unusable, analog-containing donor molecules in a cell; and that overabundance triggers a powerful feedback mechanism that dials down the production of new donor molecules—the only functional ones.

"The cell is fooled into thinking that it has enough of these donor molecules and doesn't need to make more," Rillahan said. With the combination of this shutoff signal and the analogs' physical blocking of enzymes, affected cells in the experiments soon lost nearly all the fucoses and sialic acids from their glycans.

Therapeutic Potential

One important glycan that is normally decorated with fucoses and sialic acids is known as Sialyl Lewis X. It is highly expressed on activated white blood cells and helps them grab cell-adhesion molecules called selectins on the inner walls of blood vessels. The velcro-like effect causes the circulating white blood cells to roll to a stop against the vessel wall, whereupon they exit the bloodstream and infiltrate local tissues.

The overexpression of Sialyl Lewis X or the selectins that grab this structure has been linked to chronic inflammation conditions and various cancers. Rillahan treated test cells with his best fucose and sialic acid analogs, and showed that virtually all the sialic acids and fucoses disappeared from Sialyl Lewis X molecules within a few days. Such cells were much less likely to roll to a stop on selectin-coated surfaces—suggesting that they would be much less likely to cause inflammation or cancer metastasis.

Paulson, Rillahan, and their colleagues now are working to reproduce the effects of these enzyme-inhibiting analogs in laboratory mice. "The idea is to show that these compounds can be effective in reducing the cell trafficking that contributes to inflammation and metastasis, but without harming the animals," Paulson said.

The researchers also plan to use Rillahan's screening technique to sift through large compound libraries, to try to find compounds that inhibit specific enzymes within the sialyltransferase and fucosyltranferase families. Such enzyme-specific inhibitors might have narrower treatment effects and fewer side effects than broader, family-specific inhibitors.

In addition to Paulson and Rillahan, co-authors of the paper, "Global Metabolic Inhibitors of Sialyl- and Fucosyltransferases Remodel the Glycome," are Aristotelis Antonopoulos, Anne Dell, and Stuart M. Haslam of Imperial College, London, who performed mass-spectrometry analyses to confirm the absence of sialic acids and fucoses from treated cells; Roberto Sonon and Parastoo Azadi of the University of Georgia, whose tests demonstrated the feedback-shutdown of donor molecule synthesis in treated cells; and Craig T. Lefort and Klaus Ley of the La Jolla Institute for Allergy and Immunology, who performed the cell rolling tests.

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