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Sweet Success in Targeting the Cell Surface

Howard Hughes Medical Institute researchers have successfully targeted unnatural sugar molecules with chemically unique functional groups onto the surfaces of cells in living animals without altering the animals' physiology.

The achievement is a significant advance in the promising new field of metabolic engineering because it provides a new tool for labeling specific cells in whole animals so that researchers can differentiate one cell from another.

The researchers said the new approach to marking cell-surface sugars could lead to improved understanding of fundamental cellular processes where sugars are known to play an important role, such as in interaction with pathogens, and in mediating inflammation and disease. The research may also make it possible to target the delivery of chemical agents to specific cell types in living organisms more precisely.

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- Carolyn R. Bertozzi

Led by Howard Hughes Medical Institute Medical Institute investigator Carolyn R. Bertozzi at the University of California, Berkeley, the researchers published their findings in the August 19, 2004, issue of the journal *Nature*.

"The method introduced by Bertozzi and colleagues is remarkable as a chemical process," wrote David A. Tirrell of the California Institute of Technology in an accompanying *News and Views* article in *Nature*. "The fact that specific chemical transformations can now be accomplished with spatial and temporal control in live animals is a major step forward for chemistry."

Glycosylation is the addition of carbohydrate (sugar) groups to a molecule. It has long been known that the glycosylation patterns of sugar molecules on cell surfaces can influence their interaction with other cells. “Glycobiologists have known that cancer cells, for example, exhibit changes in glycosylation patterns when compared with their normal healthy tissue counterparts,” said Bertozzi. “And there are changes in glycosylation of blood vessels at sites of chronic inflammation that are characteristic of disease. There are even some reports in changes of glycosylation in the brains of people who have prion disease or Alzheimer's disease,” she said.

Additional studies suggest that glycosylation patterns on embryonic cells may serve as developmental markers because they change as the embryo grows. Thus, studying changes in glycosylation could improve understanding of embryonic development.

But studying the role of cell surface sugar chains, called polysaccharides, in disease is best done in the context of multiple cells, said Bertozzi. “All the interesting biology we want to study takes place at the level of whole organisms. This is a general feature of glycobiology; polysaccharides exert their function largely at the systems level,” she said. This is in contrast to many proteins, such as enzymes, whose function can be studied by purifying and analyzing individual molecules.

Despite the promise of these studies, researchers faced a major challenge in finding the means to target sugars with specific markers for biological study to the surface of cells. Sugars are synthesized by complex metabolic pathways, and it was thought that integrating a marker into a specific sugar molecule would inevitably disrupt its processing in the cell.

To overcome these problems, Bertozzi and her colleagues developed a chemical technique to tag sugars in a way that does not disrupt a cell's biology and is highly specific. The technique involves “feeding” a cell a slightly modified sugar with a chemical group called an azide attached. Such sugars are not normally found on cells, but are processed by the cell's metabolic pathways similarly to normal sugars and are incorporated into the cell-surface polysaccharides. The researchers can then tag the resulting “azido sugar” on the cell surface by treating it with a molecule called a phosphine to which any desired molecule, such as a probe for visualization, can be attached.

This reaction, called the Staudinger ligation, is “bio-orthogonal,” said Bertozzi—meaning that it does not affect the cell's biology; and the components form a covalent bond with one another in a highly selective manner.

In the research reported in *Nature*, Bertozzi and her colleagues describe the first use of their cell-surface engineering technique in living animals. Previously, they had only applied it to cultured cells.

They injected the azido sugar into mice and used the Staudinger ligation to attach a phosphine molecule that carried a distinctive tag that would enable the scientists to detect whether attachment to the cell surface had occurred.

The researchers found that the azido sugar made its way into the mouse organs, was chemically processed similarly to the normal sugar, and appeared on the cell surface. They also found that the unnatural sugar caused no adverse physiological effects, even at the largest doses.

“We weren't particularly surprised at the lack of toxicity because unnatural sugars are not known for high toxicity,” said Bertozzi. “And at the highest dose, the amount of sugar we gave the animals was about that contained in a can of soft drink. Also, the azide component is already used in clinically approved drugs, such as AZT, which is taken at much higher dosages,” said Bertozzi.

The scientists' analyses revealed that the azido sugars were most concentrated in the heart, kidney and liver, with much lesser amounts in the brain and thymus. These findings indicate it may be able to apply this tagging technique to study the biology of other organs and to look for changes in organs that occur in diseases as cancer.

According to Bertozzi, advancing the technique to living animals will have important research and clinical implications. “From our point of view, one of the most exciting implications of this work is the prospect for imaging glycosylation in real time within living organisms,” she said. “We hope to be able to witness changes in the pattern of glycosylation in a tissue as an animal develops through the embryonic stages, as a disease develops, or as tumors become metastatic. Until now, there has not been a technique to do such imaging.”

Bertozzi and her colleagues are working on probes that could be attached to a phosphine, including those that can be used in magnetic resonance imaging, positron emission tomography and single photon emission computed tomography. They are also developing new bio-orthogonal ligation reactions with azides that will give them additional sugar-tagging techniques.