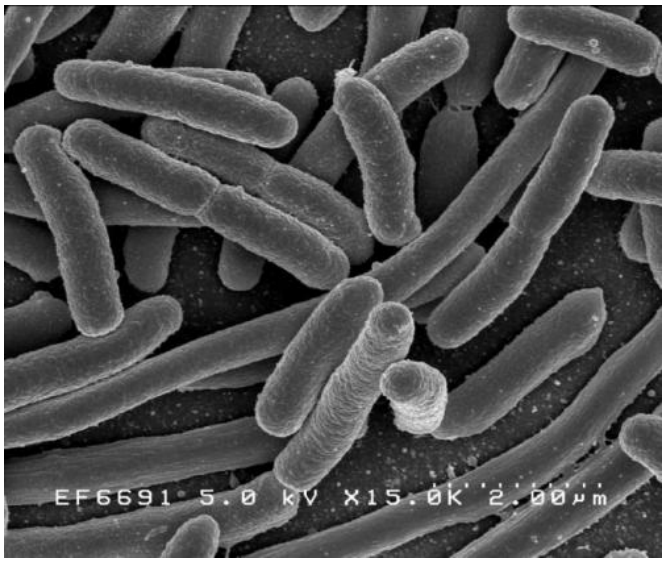
Engineered bacteria churn out cancer biomarkers

17 September 2020



Escherichia coli. Credit: Rocky Mountain Laboratories, NIAID, NIH

Pity the glycan—these complex sugar molecules are attached to 80% of the proteins in the human body, making them an essential ingredient of life. But this process, known as glycosylation, has been somewhat overshadowed by flashier biomolecular processes such as transcription and translation.

"Glycosylation is absolutely essential for life on this planet. And yet, we still know relatively little about it," said Matthew DeLisa, the William L. Lewis Professor of Engineering in the Smith School of Chemical and Biomolecular Engineering. "While much attention has been given to understanding the genome and the proteome, the glycome—which represents the entire complement of sugars, either free or present in more complex molecules such as glycoproteins, of an organism—has been relatively understudied. We need new tools to advance the field forward."

DeLisa's lab has created these very tools by commandeering simple, single-celled microorganisms—namely *E. coli* bacteria—and engineering them to explore the complex process of glycosylation and the functional role that protein-linked glycans play in health and disease.

The group's paper, "Engineering Orthogonal Human O-linked Glycoprotein Biosynthesis in Bacteria," published July 27 in *Nature Chemical Biology*. The lead author is Aravind Natarajan, Ph.D. '19.

Previously, DeLisa's team used a similar cell glyco-engineering approach to produce one of the most common types of glycoproteins—those with glycan structures linked to the amino acid asparagine, or N-linked. Now the researchers have turned their attention to another abundant glycoprotein, namely O-linked, in which glycans are attached to the oxygen atom of serine or threonine amino acids of a protein.

The O-linked glycans are more structurally diverse than their N-linked cousins, and they have important implications in the development of new therapeutic treatments for diseases such as breast cancer.

"Our cell-engineering efforts were quite complicated as we not only needed to equip *E. coli* with the complete set of enzymes for making and attaching glycan structures to proteins, but we also had to carefully rewire native metabolic networks to ensure the availability of important glycan building blocks such as sialic acid," Natarajan said. "The addition of sialic acid to our glycoproteins is significant because this sugar residue is often crucial for targeting drugs to specific cells and increasing their circulatory half-life."

When a cell turns cancerous, it expresses certain biomarkers, including abnormally glycosylated surface proteins, that indicate the presence of cancer. DeLisa's group equipped *E. coli* with the

machinery to produce such proteins, including one that closely resembled a prominent cancer biomarker, mucin 1 (MUC1).

"The glycosylated version of MUC1 is one of the highest-priority target antigens for cancer therapy. It's been very challenging to develop therapies against this target," said DeLisa, the paper's senior author. "But by having a biosynthetic tool like the one we've created that is capable of replicating the MUC1 structure, we're hopeful that this could provide glycoprotein reagents that could be leveraged to discover antibodies or employed directly as immunotherapies, all of which could help in the fight against certain types of cancer."

Both O-linked and N-linked glycans have also been discovered in one of the surface proteins of the SARS-CoV-2 virus, which causes COVID-19. DeLisa is hopeful his group's method of bacterial cell glyco-engineering will open the door for creating glycosylated versions of this S-protein that could lead to therapeutic antibodies against the coronavirus, or the development of a subunit vaccine.

Because of their earlier work replicating N-linked glycans, the researchers were able to get the O-linked system up and running quickly. Now DeLisa's lab is primed to make proteins that carry both types of glycosylation, which is significant because many glycoproteins, such as the S-protein in SARS-CoV-2, carry both N- and O-linked glycan structures.

The researchers are also exploring ways to increase the spectrum of glycoproteins that their engineered *E. coli* cells can produce and the efficiency with which these products are generated.

"We think of *E. coli* as a clean chassis or a blank slate when it comes to protein glycosylation, because these bacteria do not normally peform glycosylation reactions like the ones we have installed," DeLisa said. "This allows construction of these pathways from the bottom up, giving us total control over the types of glycan structures that are made, and the specific sites in target proteins where they're attached. That is a level of control that is difficult to achieve with other preexisting cell-based systems or technologies for glycoprotein engineering."

More information: Aravind Natarajan et al, Engineering orthogonal human O-linked glycoprotein biosynthesis in bacteria, *Nature Chemical Biology* (2020). DOI: 10.1038/s41589-020-0595-9

Provided by Cornell University