PROTEOMICS

Snapshots of the cell surface

Researchers develop an approach to selectively isolate and profile cell-surface proteins by targeting the glycopeptides, a strategy that could be used to generate an atlas of cell-surface protein 'barcodes'.

Isolating cell-surface glycopeptides. Glycans are selectively oxidized, tagged with biocytin hydrazide, the glycoproteins are digested, the glycopeptides are affinity-purified, and finally the peptides are released for mass spectrometry analysis. Reprinted from *Nature Biotechnology*.

What defines a cell type? One way to distinguish cell types is by taking advantage of the unique markers found on their surfaces. Traditionally, antibodies have been used for this job. But for the majority of cell-surface proteins, suitable antibodies are not available and only a small number of antibodies can be applied in parallel.

Mass spectrometry–based proteomics technologies can pick up where antibodies fall short. With mass spectrometry, researchers can look at hundreds or thousands of proteins at once, even obtaining quantitative information. The technology is powerful, but often the bottleneck for researchers is in obtaining a clean biochemical preparation of the proteome of only the system of interest.

Bernd Wollscheid of the Institute of Molecular Systems Biology at the Swiss Federal Institute of Technology and Julian Watts of the Institute for Systems Biology in Seattle, together with their team, now describe a strategy to profile the cell-surface proteome. Previous efforts to analyze the cell-surface proteome have met severe challenges in isolating membrane protein fractions and in analyzing hydrophobic transmembrane domains by mass spectrometry. But Wollscheid's group had what he calls a "'sweet' inspiration." "We thought about what's different about the cell surface, and there's definitely one thing that is special, which is that most proteins at the cell surface are glycosylated," he says. If they could selectively capture the glycosylated peptides at the cell surface, they reasoned, then profiling via mass spectrometry would be relatively straightforward.

The cell-surface capturing (CSC) strategy they developed to isolate surface N-linked glycopeptides on intact, living cells involves first using a mild oxidation procedure to convert the *cis*-diol groups found on all glycans to an aldehyde and then labeling with biocytin hydrazide. The labeled peptides are digested and affinity purified, via the biotin moiety, on streptavidin beads. They then thoroughly wash the beads to remove any contaminants and ensure a clean glycopeptide fraction. The peptides are finally released from the beads using an enzyme that snips the glycan off the asparagine residue to which it is attached. In the process of cleavage, asparagine is converted to aspartic acid. This introduction of a mass shift serves two purposes: it can be detected by the mass spectrometer to confirm both the location of the glycosylation site and also that the formerly glycosylated peptide originated from the cell surface.

Wollscheid's group has used CSC to profile the cell-surface proteomes of several different cell lines as well as primary cells and tissues. They also have combined CSC with quantitative proteomics approaches. In one application, they looked at changes to the T-cell surface glycoproteome as a result of T-cell activation. In another interesting example, they followed how the surface proteome of mouse embryonic stem cells changed as they differentiated into neural progenitor cells. "The question for us is, is the cell different because it has different proteins exposed on the cell surface, or is the cell different because it has the same proteins but in different quantities?" says Wollscheid. "We now have a technology to follow this." They are currently working on developing an absolute quantitative approach by using proteotypic (unique to a particular protein) peptides as standards, which should greatly increase the sensitivity of the method.

Though proteomics detractors may be tempted to call such experiments 'catalog science', "at the same time, there wasn't much knowledge about the proteins at the cell surface," explains Wollscheid. "You could spend months and months in the library figuring this out [for a cell of interest] and would probably end up with only a limited amount of information." Systems biology experiments require multiplexed, quantitative measurements of protein nodes in specific locations over time to reconstruct cellular networks; CSC makes these experiments possible for cell-surface proteins. The group is using their proteomic data to establish a cell-surface protein 'barcode' atlas, which they call Sisyphus. The unique quantitative marker panels they are identifying for different cell types also show promise for the selection and development of new antibodies.

Wollscheid is particularly interested in using the approach to define subtypes of cells of clinical interest, for example, brain cells and brain cancer cells, especially where molecular markers are lacking. But he also sees the approach as being very useful for addressing basic biological questions. "We now have a possibility to really look at cell-surface proteins simultaneously," he says. "We can now ask how the cell-surface subproteome is changing upon various perturbations, such as drug treatment, and that's the next question we are about to address."

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RESEARCH PAPERS

Wollscheid, B. *et al.* Mass-spectrometric identification and relative quantification of N-linked cell surface glycoproteins. *Nat. Biotechnol.* **27**, 378–386 (2009).