Q&A: How single-cell and spatial proteomics reveal proteins' nuanced roles in health and disease

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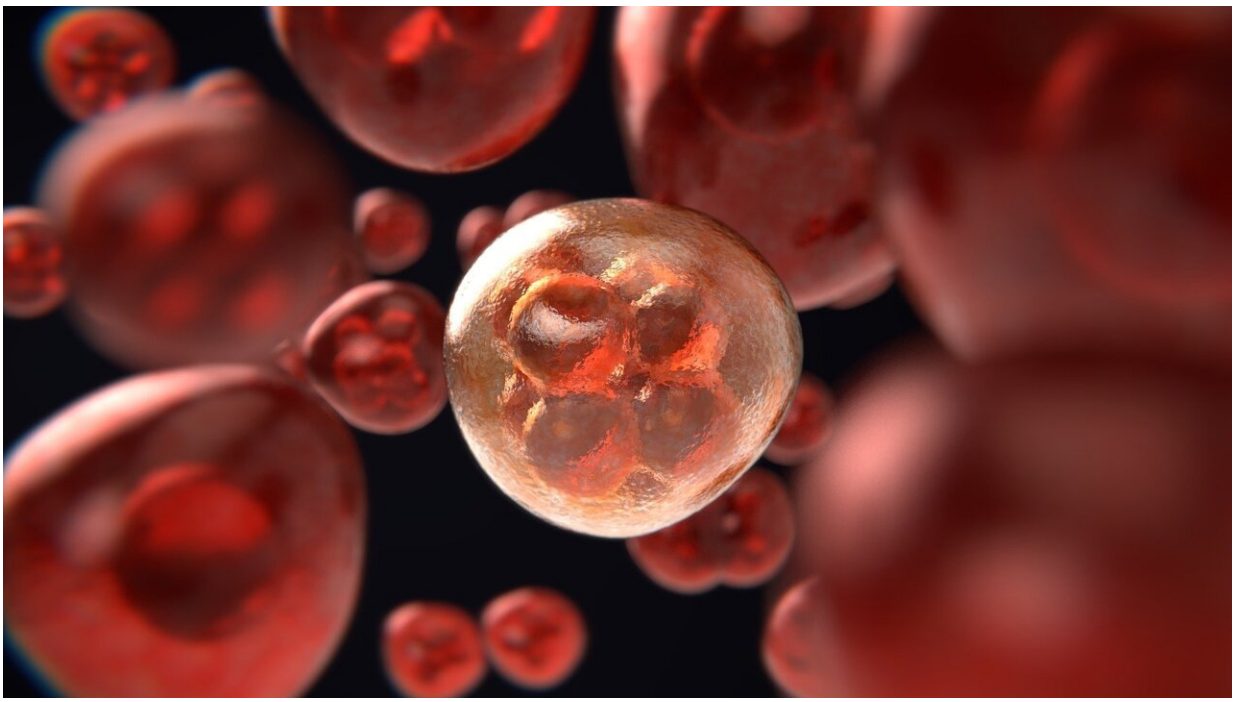

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When Steve Carr, senior director of the Proteomics Platform at the Broad Institute of MIT and Harvard, began working in proteomics, the field was able to detect only the most abundant proteins in a given sample. In recent years, increasingly sensitive mass spectrometers have

paved the way for enormous progress: scientists are now able to detect and analyze nearly all proteins expressed in a sample, including ones present only in miniscule amounts.

Recent improvements in sample handling and mass spectrometer sensitivity are now enabling further advances, in the form of single-cell and spatial proteomics technologies. These methods can identify proteins in individual cells, rather than bulk tissue, and map the location of specific proteins within a tissue, allowing scientists to learn more details about how cells function and communicate. This progress is in part driven by optimized methods and more powerful tools developed by researchers such as Claudia Ctortecka, a postdoctoral associate in the Proteomics Platform headed by Carr, and colleagues in the Platform.

Carr and Ctortecka are seeking collaborations to apply new single-cell and spatial proteomics technologies to ever more complex questions. They hope to push the technology further and use it in combination with tools from other fields to learn about the full range of cellular activity, from gene expression to post-translational modifications.

We spoke with Carr and Ctortecka about the potential of spatial and single-cell proteomics and their hopes for future collaborations.

What can single-cell and spatial proteomics now enable?

Carr: In the past, proteomics used bulk material—samples consisting of millions of cells—all lysed together and then profiled at the proteome and post-translational modification level. In doing that, we lost information about the uniqueness of each cell, and we had limited information about the spatial heterogeneity of tissues that plays a decisive role in disease.

Single-cell proteomics technologies with an analysis depth of thousands of proteins quantified per cell have only recently emerged. It's also only recently become possible to develop spatial profiling methods capable of deep-scale, unbiased proteome analysis in defined regions of tissue.

Ctortecka: Earlier in the field's history, we were mostly monitoring cell identity, looking for the most abundant proteins that describe what type of cell we're talking about. Now we're getting more and more sensitive. We can find subpopulations of a specific cell type that we're interested in. We can see transition states; we can see cells that are morphologically very different. And we can derive meaningful biological information by doing this.

We're also now able to do better and more reproducible protein quantitation, which is key. The presence of one protein alone doesn't tell you much about a cell state. It's a matter of how much of that protein is present relative to its environment. The relative difference between cell A and cell B might give you an idea of why one cell reacts to a treatment and the other cell does not.

With these new approaches, what kinds of more complex questions can scientists ask?

Carr: Cells that are near one another talk to each other. Cells have a secretome: secretions from cell A trigger phenomena in cell B. In order to study that, we need to be able to look at single cells, and we need to have spatial information about them. We're interested in studying how what's happening in cell populations in one region of a tissue influences a cell population in a different region of the same tissue.

Ctortecka: When looking at spatial resolution, we're looking at multiple cells in context. We can start to narrow in on cells that look similar, that

are located next to something interesting, or that are part of a tissue that's interesting. This helps us understand the signaling happening within a tissue and how differences in protein abundance play a role. We can start to extrapolate, for example, why some patients are more resistant or sensitive to treatment.

Single-cell genomics has really transformed our understanding of how cells work in health and disease. Do you think single-cell and spatial proteomics will have a similar kind of impact?

Ctortecka: Proteomics has always been a complementary approach to other -omics strategies, and I believe this will hold true again. The genomics or transcriptomics information we're able to obtain across thousands of cells from a tissue provides invaluable insights into the presence of subpopulations, and possibly their identity markers.

With the ability to select for those subpopulations using fluorescence sorting or laser-capture microdissection, we can profile their proteome with high resolution, providing another layer of information. Proteomics currently cannot compete with the throughput of sequencing strategies, but I'm convinced that single-cell and spatial proteomic information will make its way into the clinic and help us provide the missing piece to understand how cells actually make decisions.

What kinds of collaborations are you looking for, and what is an example of a current one?

Ctortecka: We want to show the Broad community what we can do, and we want to hear from the Broad community what they want to know. We're really interested in pushing the technology further in order to answer biological questions. Tailoring our approach based on these biological questions is something we're really excited to do and

something we're becoming very good at.

For example, we're working on a collaboration with Melina Claussnitzer looking at temporal cell differentiation. We have a cell population very early in development that is being pushed into a specific niche, and we can profile it at single-cell resolution. It's the first time this has ever been done on the proteome level, and we couldn't have come up with this approach without our collaboration partner.

Carr: We're learning a lot through these collaborations. There are still challenges, but we're at a point now where a lot of these things are not physical barriers. They're engineering problems that we just need to find a solution to.

What are you currently working on to develop these technologies further?

Ctortecka: We're interested not just in the protein composition of a cell, but also in post-translational modifications at the single-cell level, because those may be more indicative of a cell state and are key to subcellular and intercellular signaling.

Carr: Analyzing protein modifications has been a long term interest of mine, and it is an area that the group has focused on since we formed back in 2004. We've developed global methods for analyzing a wide range of post-translational modifications—including phosphorylation, ubiquitylation, and acetylation—from the same sample using serial enrichment strategies. Adapting these methods to spatial or single-cell samples is one of our current challenges. In a recent study we successfully profiled a wide range of histone modifications at the single-cell level.

One of the next challenges for us is looking at phosphorylation and changes in signaling pathways in defined regions of tumor tissue. Characterizing well-described functional phosphorylation patterns and the kinases involved can provide insights to clinically relevant biology.

Ctortecka: Another challenge is developing informatics that can confidently identify and quantify proteins or post-translational modifications with minimal sample input. Interpreting the complex data we generate with our increasingly fast and sensitive mass spectrometers requires a lot of expertise, since analysis software increasingly relies on machine learning. The field started out using machine learning to distinguish between true positives and false positives, but now we're using it to predict diverse parameters and evaluate how confident our identifications are.

Carr: The advances that have occurred over just the past few years in the field of proteomics, while astonishing, are just a prelude to what is likely to come over the next few years.

Provided by Broad Institute of MIT and Harvard

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