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AMIR GILADI & IDO AMIT

Single-cell genomics can identify unique immune cells (red) involved in Alzheimer's disease.

Immunology, one cell at a time

Analysing the DNA, RNA and protein of single cells is transforming our understanding of the immune system, say **Amir Giladi** and **Ido Amit**.

For more than a century, scientists have tried to characterize the different functions of the 10 trillion to 50 trillion cells of the human body — from neurons, which can reach 1 metre in length, to red blood cells, which are around 8 micrometres wide. Such efforts have helped to identify important cell types and pathways that are involved in human physiology and pathology.

But it has become apparent that the

research tools of the past few decades fail to capture the full complexity of cell diversity and function. (These tools include fluorescent tags fused to antibodies that bind to specific proteins on the surfaces of cells, known as cell-surface markers, and sequencing in bulk of the RNA or DNA of thousands of seemingly identical cells.) This failure is partly because many cells with completely different functions have similar shapes or

produce the same markers.

Single-cell genomics is transforming the ability of biologists to characterize cells. The new techniques that have emerged aim to capture individual cells and determine the sequences of the molecules of RNA and DNA that they contain. The shift in approach is akin to the change in how cells and molecules could be viewed during the 1980s, following advances in microscopy and the tagging and sorting of cells.

In the past five years, several groups of biologists, including our own laboratory, have gone from measuring the expression of a few genes in a handful of cells to surveying thousands of genes in hundreds of thousands of cells from intact tissues. New cell types^{1,2}, cellular states and pathways are being uncovered regularly as a result.

Our lab was one of the first to study the immune system using single-cell genomics. The tools are particularly suited to this task because the heterogeneity and plasticity of cells are integral to how the immune system works — the nature of each agent that could attack the body being impossible to know ahead of time.

Exploiting single-cell genomics fully will require scientists and clinicians to make experimental and analytical adjustments. In particular, we must be ready to jettison assumptions about cell types and cellular states, and to rebuild representations of cellular networks.

BLUNT INSTRUMENT

The cells of the immune system, which patrol the blood and dwell in tissues, have many functions. They protect the body from pathogens and cancer, and orchestrate metabolism and the formation of organs. They are involved in almost every activity that regulates the body's internal environment, from the development and remodelling of tissues to the clearance of dying cells and debris. So their dysfunction can cause many problems. For instance, deregulated immune cells can attack healthy cells and cause autoimmune conditions such as lupus, type 1 diabetes or multiple sclerosis. ▶

SINGLE-CELL BIOLOGY

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► A first step towards harnessing the immune system for therapeutic use is to characterize the types of cell that occupy a specific area (such as the surroundings of a tumour). Another is to map the unique processes and pathways that the cells are involved in, the genes they express and the cells' interactions and responses to environmental cues. Over the past 40 years, meticulous approaches based on genetic labelling have enabled researchers to identify dozens of types and functions of immune cells. For example, the use of antibodies fused to fluorescent tags that bind to and flag specific cell-surface markers established the basic taxonomy of immune cells — including several types of T cells, B cells, monocytes and granulocytes. Such studies also kick-started the search for treatments known as immunotherapies, which harness the body's natural defences to fight disease.

Increasingly, however, these techniques hint that the world of immune cells is more complex than current categories allow. Immune cells seem to change their functions depending on their surroundings. For instance, macrophages (as identified by their cell-surface markers) might have one function in the gut yet a completely different one in the brain³. Also, molecular markers cannot fully describe the functional diversity of cells in different immune contexts. For example, a group of immune cells that suppresses the immune response around tumours (myeloid-derived suppressor cells) has been shown to express markers from both monocytes and granulocytes⁴.

In short, conventional methods based on populations of cells are proving too blunt a tool with which to tease apart complex immune assemblies⁵.

CLOSE READING

In the past five years, technologies for capturing single cells have improved dramatically. Some approaches rely on placing cells inside miniature vessels, one at a time; others capture individual cells inside droplets of oil. Meanwhile, bioinformaticians have built algorithms for representing multidimensional data, identifying distinct cellular states and modelling the transitions between such states⁶.

Thanks to these developments, researchers can now capture hundreds of thousands (or even millions) of cells and accurately measure the DNA, RNA or protein content of each (see 'Scale up'). Gene-editing tools such as the CRISPR–Cas9 system can be used to introduce a specific mutation into the genome of one cell, and then a different alteration into the next⁷. Thus, the function of dozens of genes can be inferred from just one experiment by reading the resulting RNA 'barcode' in parallel with the single-cell genetic information.

With such measurements, researchers can potentially record the functional states of many cells at once⁸. They can also probe the ancestry of individual cells⁹ or identify mutations in a particular cell's DNA, as well as track communication between cells. In other words, single-cell genomics allows researchers to build an accurate representation of the entire make-up of a tissue¹⁰, such as a specific organ or a tumour, or of a multicellular process such as the immune system's response to an infection. Importantly, it enables them to do this without making prior assumptions based on, for instance, the participating cell types and their characteristics.

About 20 labs worldwide have fully embraced single-cell genomics, and even more are trying out the approach. In the past few years, numerous papers have been published that describe new types of immune cell and previously unknown pathways involved in various conditions.

For instance, 15 subtypes of innate lymphoid cell, which are similar to T cells but do not express the T-cell receptor, have been identified in the gut¹¹. Different progenitors of immune-cell lineages have been uncovered in the bone marrow¹². Specific types of immune cell have been associated with particular stages of tumour growth^{13,14}. And various types of microglial cell have been identified in the brain during development.

Last month, our lab reported the discovery of a new type of immune cell in the brain, disease-associated microglia (DAM)¹⁵. Our experiments indicate that DAM break down dead cells and protein aggregates called plaques in the brains of mice engineered to express mutated proteins associated with

Alzheimer's disease.

More than a decade of population-based assays, including cell sorting using specific cell-surface markers and bulk RNA sequencing, had failed to flag these cells. Only by individually sequencing the RNA of each of the immune cells in a sample of brain were we able to find a rare subpopulation of microglia that may open up fresh approaches to treating Alzheimer's disease.

BASIC LESSONS

It is early days for single-cell genomics. But already, a number of important lessons can be learnt from the experiences of our lab and those of others.

First, it is clear that many of the current categories of immune cells, such as T cells or monocytes, encompass heterogeneous populations. To probe cellular complexity, researchers must therefore cast their nets wide, and try to collect all immune cells within a tissue or region of interest. This is a very different approach from that used with methods based on cell-surface markers, which aim to obtain as pure a sample as possible.

Second, success will depend, in part, on the extent to which researchers preserve the states of cells and the original composition of a tissue. Cell stress or death should be minimized to ensure that tissue preparation does not favour specific cell types. (Some are more sensitive to heat stress, for example, than others.)

Third, bioinformaticians will need to develop scalable and robust algorithms to cope with greater numbers of cells, conflicting or overlapping programs of gene expression and fleeting developmental stages.

Fourth, after researchers have characterized all of the immune cells in a sample, they will need to find molecular markers that can be used to either enrich or deplete certain cell types in further samples. Tissues comprise trillions of cells with myriad molecular characteristics and functions, and the types or states of these cells may vary in abundance by many orders of magnitude. For instance, in the brains of healthy mice, our newly identified population of DAM makes up less than 0.01% of cells¹⁵. Thus, repeated unbiased sampling to characterize rare populations will keep on accumulating cells that are not those of interest.

Other experimental, computational and statistical approaches can help to overcome this problem. Importantly, once a rare population of cells is identified using single-cell genomics, they can be purified and experiments conducted only on them. In our recent study, for instance, we used cell-surface markers to isolate DAM and then assessed their role in Alzheimer's disease using various techniques, including fluorescent labelling.

"Single-cell genomics will soon be commonplace in basic and applied immunology research."

SCALE UP

In the past decade, biologists have moved from analysing a few genes in a handful of cells, one cell at a time, to surveying thousands of genes in hundreds of thousands of cells, in parallel.

*Data points included represent high-profile studies that introduced technological advances in single-cell RNA sequencing.

GENETIC MICROSCOPE

By analysing the DNA, RNA and protein content of immune cells — one cell at a time — biologists can build a more accurate representation of a tissue or tumour's cellular make-up than conventional tools allow.

PROLIFERATING CELLS

Some immune cells, including T cells, proliferate in the environment that surrounds a tumour.

GRANULOCYTE

A type of innate immune cell that attacks invading pathogens or tumour cells.

ENGULFING CELLS

Some immune cells such as macrophages engulf and digest cellular debris and tumour cells.

ANTIGEN-PRESENTING CELL

An immune cell that displays fragments of pathogens (antigens) for recognition by other cells.

DIFFERENTIATING CELLS

The pattern of gene expression and the function of immune cells can change dramatically in and around the tumour.

OLD VERSUS NEW

Mixture of immune cells.

Cell identity is lost.

BULK RNA SEQUENCING

Sequencing a mixture of seemingly identical cells fails to capture the diversity of the immune cells surrounding a tumour.

SINGLE-CELL GENOMICS

Using single-cell genomics, biologists can capture the molecular signature of all immune cells found in and around the tumour.

Such maps would allow biologists to define cells not just by one fate, lineage or function, but by the combination of all of these. It would also allow these functional entities to be compared across organisms.

RAPID TRANSFORMATION

Single-cell genomics will soon be commonplace in basic and applied immunology research. This is thanks to efforts to make the tools affordable, standardized and accessible to academia, the biotech industry and the clinic. We predict that, within a decade, blood samples or biopsies will be routinely sent for single-cell genomic analysis, and the entire immune composition of patients analysed and compared with all known healthy and diseased states.

Also likely to undergo rapid transformation is our understanding of tumours and tumour stem cells, processes such as neuronal development, metabolic disorders and neural function.

Almost every scientific breakthrough has originated from a new measurement or observation that enabled scientists to come up with new hypotheses and merge them into unifying theories. Robert Hooke's observation of cells as units of multicellular organisms, James Watson and Francis Crick's discovery of the 3D structure of DNA and Edwin Hubble's detection of galaxies beyond the Milky Way could not have been achieved without new ways of seeing.

The molecular microscope of single-cell genomics is already adding to our knowledge of cell types and gene pathways. But for single-cell genomics to tell us something truly new about the blueprint of humans, we will have to address how individual cells communicate to achieve shared goals. ■

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A fifth lesson regards a considerable drawback of current single-cell technologies. They capture snapshots of dynamic systems, in which cells are devoid of important context — spatial, temporal, clonal and epigenetic. Without knowing where a profiled cell came from, who its neighbours were or what it developed from, it is hard to model complex processes such as tissue formation or a tumour's interaction with nearby immune cells.

One way around this problem might be to combine several layers of information from the same cell. Genetic fluorescent labelling, for instance, can be used to track changes in the state of a cell over time or to find exactly where it is in a tissue.

Ultimately, textbook definitions and long-held beliefs about cellular identities,

such as the distinction between cell type and cell state, will almost certainly need to be rethought. Some classifications of subgroups based on extra markers may prove helpful in the short term, but can quickly become unwieldy. For example, instead of being able to refer to T-helper (T_H) cells, researchers must now refer to one of about a dozen subcategories, including T_H1 cells and T_H2 cells¹⁶. And such an approach may continue to overlook the true functional complexity of the immune ecosystem.

A more workable solution may be for researchers to replace rigid classifications with assemblies of gene-expression programs (see 'Genetic microscope'). These elaborate gene maps could represent all cell types and states, including those from different physiological and pathological contexts.