


Mireia Ramos-Rodríguez, Lorenzo Pasquali.
Genomic Endocrine Regulation, Department of Medicine and Life Sciences, Universitat Pompeu Fabra (UPF), Barcelona, Spain.
Members of the Working Group on Basic Experimentation in Diabetes, Spanish Society of Diabetes (SED).

New Techniques for Studying Genomics in Type 1 Diabetes

Type 1 Diabetes (T1DM) is a multifactorial disease resulting from a complex interplay of genetic and environmental factors, leading to the autoimmune destruction of beta cells in the pancreas. Although T1DM does not follow a Mendelian inheritance pattern, it has a clear genetic component that predisposes certain individuals to T1DM disease. T1D involves multiple cell types, such as autoreactive T lymphocytes attacking beta cells, as well as various immune and non-immune pancreatic cells. This genetic and cellular complexity demands an integrated approach to understanding pathogenic mechanisms and developing effective strategies for early diagnosis and treatment.

Omics sciences study complete sets of molecules within an organism, such as transcriptomics (RNA) and proteomics(proteins), offering a holistic view of biological networks and how these molecules interact and regulate biological processes. Genomics, in contrast, focuses on the study of an organism's complete genome, including the sequencing, analysis, and interpretation of its genetic material. This field encompasses the identification of genes and their sequences, as well as their contribution to the structure, function, and evolution of the genome.

A better understanding of the genomic aspects related to T1DM offers several key benefits. It enables early diagnosis by identifying genetic markers associated with disease predisposition, facilitating monitoring and early intervention. Moreover, advances in genomics enable personalized medicine by adjusting treatment according to each patient's genetic profile, thereby improving efficacy and reducing side effects. Finally, a deep understanding of the genetic basis of T1DM drives the development of new therapies by identifying specific targets for intervention and designing treatments that address underlying genetic alterations.

TECHNIQUES FOR SINGLE-CELL GENOME STUDY

Historically, the study of different omics fields has relied on groups of cells, such as tissue samples or multiple pancreatic islets. The resulting signal is an aggregate of signals from all the cells, and in the case of a diverse population, cellular heterogeneity is masked ***(Figure 1)***.

There are techniques that can identify and isolate a cell type of interest. One such method is fluorescence-activated cell sorting (FACS), which allows cells to be counted, »

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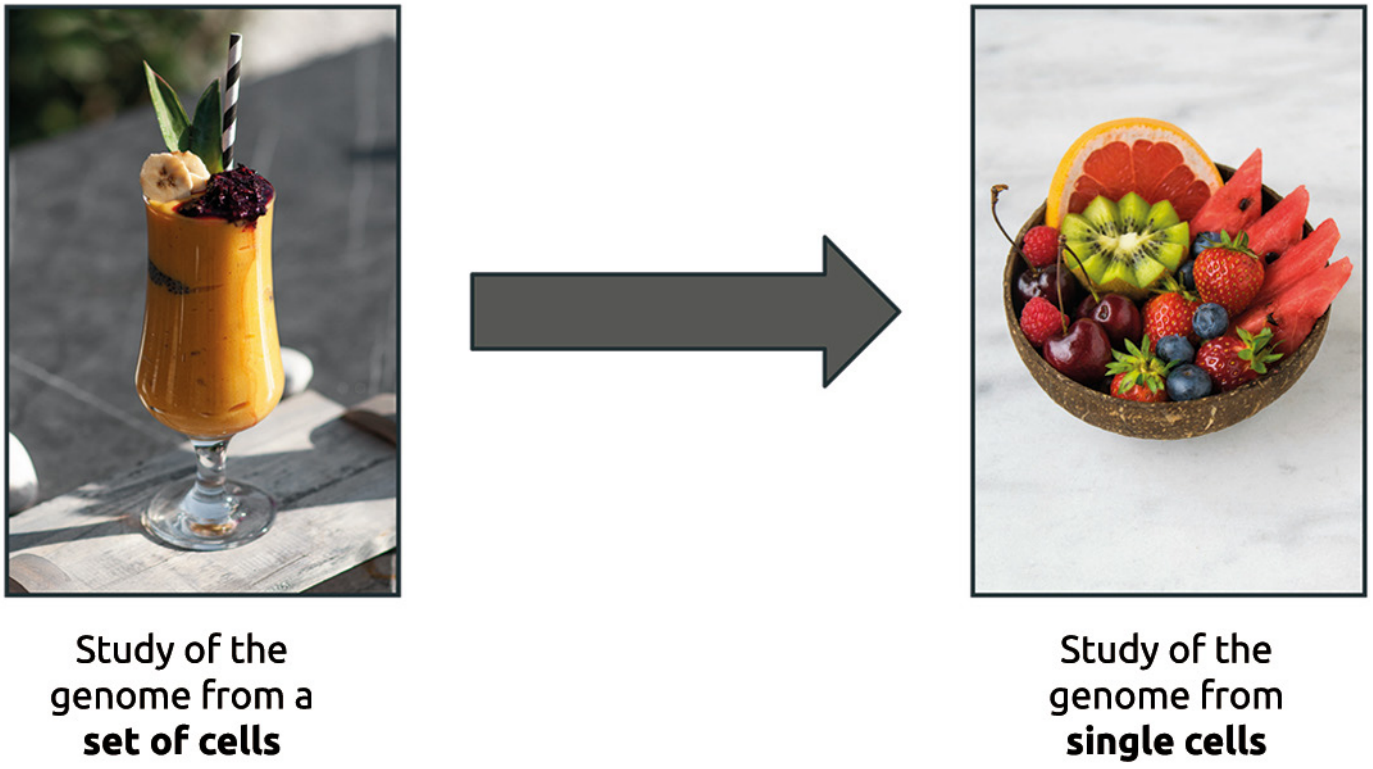

FIGURE 1. Comparison between studying the genome from a group of cells (juice), where the signal between different cell types is indistinguishable, and from single cells (fruit bowl), where we can identify the contribution of each cell individually. Photos by Kim Ick and Jo Sonn on Unsplash.

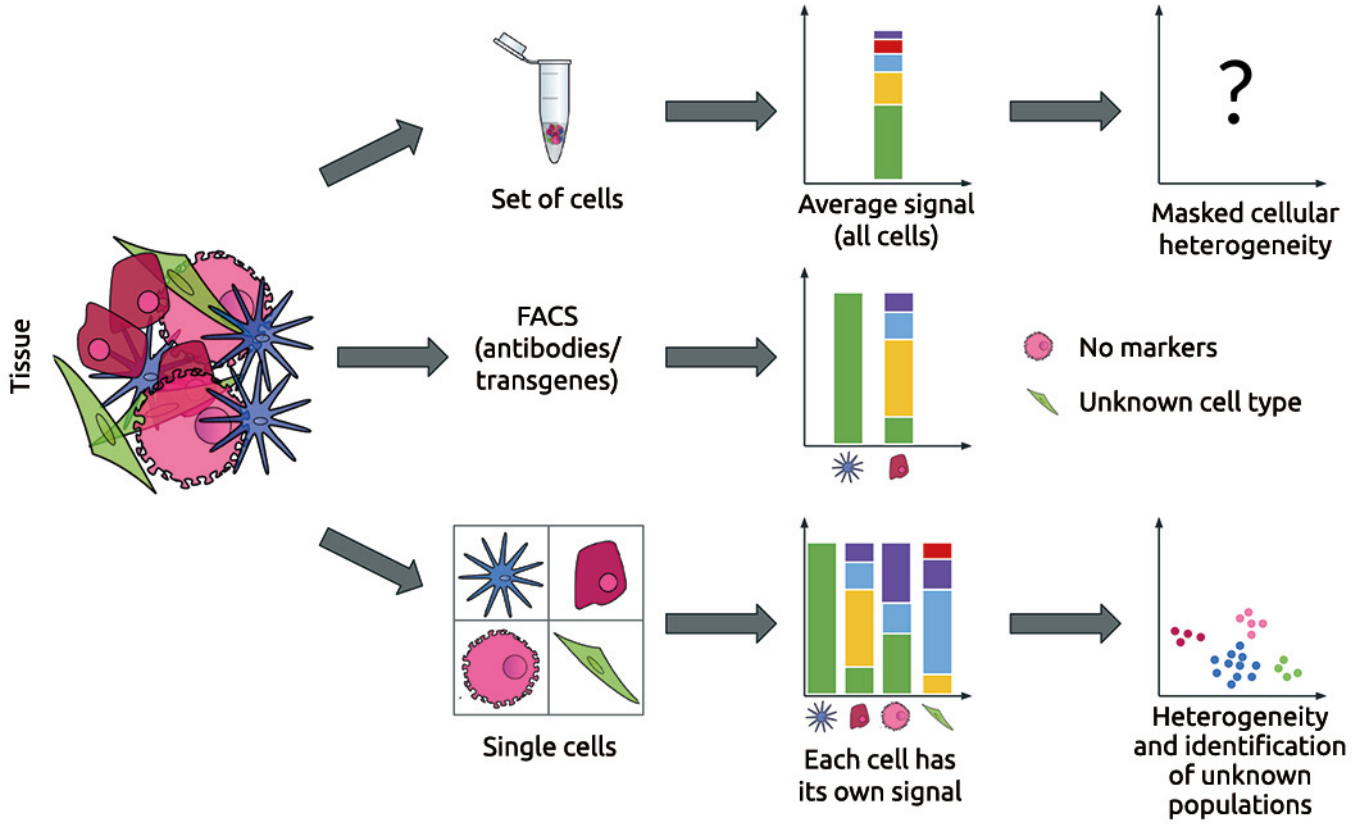

FIGURE 2. Description of different technologies for obtaining omics data from tissues or cell populations.

» classified, and analyzed individually within a complex mixture. This is achieved by using transgenes or antibodies labeled with fluorochromes that bind to specific proteins on the cell surface. A limitation of this technique is the requirement for prior knowledge of markers that can identify the cell type of interest. Thus, it is not possible to select cell types for which markers are unknown or to identify previously undescribed cell types.

In this context, the use of single-cell technologies allows for the independent characterization of each cell within a tissue. This enables researchers to analyze the degree of heterogeneity and identify new cell types or states without relying on markers or prior knowledge ***(Figure 2)***.

STRATEGIES FOR OBTAINING SINGLE CELLS

In 2009, the transcriptome of a single cell was analyzed for the first time[1]. From this milestone, the challenge shifted to developing strategies to obtain this information from an increasing number of cells. Over the last decade, single-cell technologies have undergone significant evolution, advancing from characterizing only a few cells to studying the cellular composition of entire organisms[2]. Currently, studies typically analyze tens of thousands to hundreds of thousands of cells in large-scale research.

Various strategies have been developed to isolate or identify cells individually, each offering different levels of sensitivity and enabling the characterization of varying numbers of cells[3]. These strategies can be categorized into three main groups based on the method of single-cell separation: 1) Tubes, microfluidics, plates, or chips; 2) Droplets; 3) Molecular barcodes or indexing

Separation via tubes, microfluidics, plates, or chips. In this approach, individual cells are distributed into separate wells or tubes, where libraries—a collection of molecules to be identified—are prepared. Each library receives a molecular identifier for the molecules originating from the same cell. This technology is suited for analyzing a limited number of cells but produces more complex libra- »

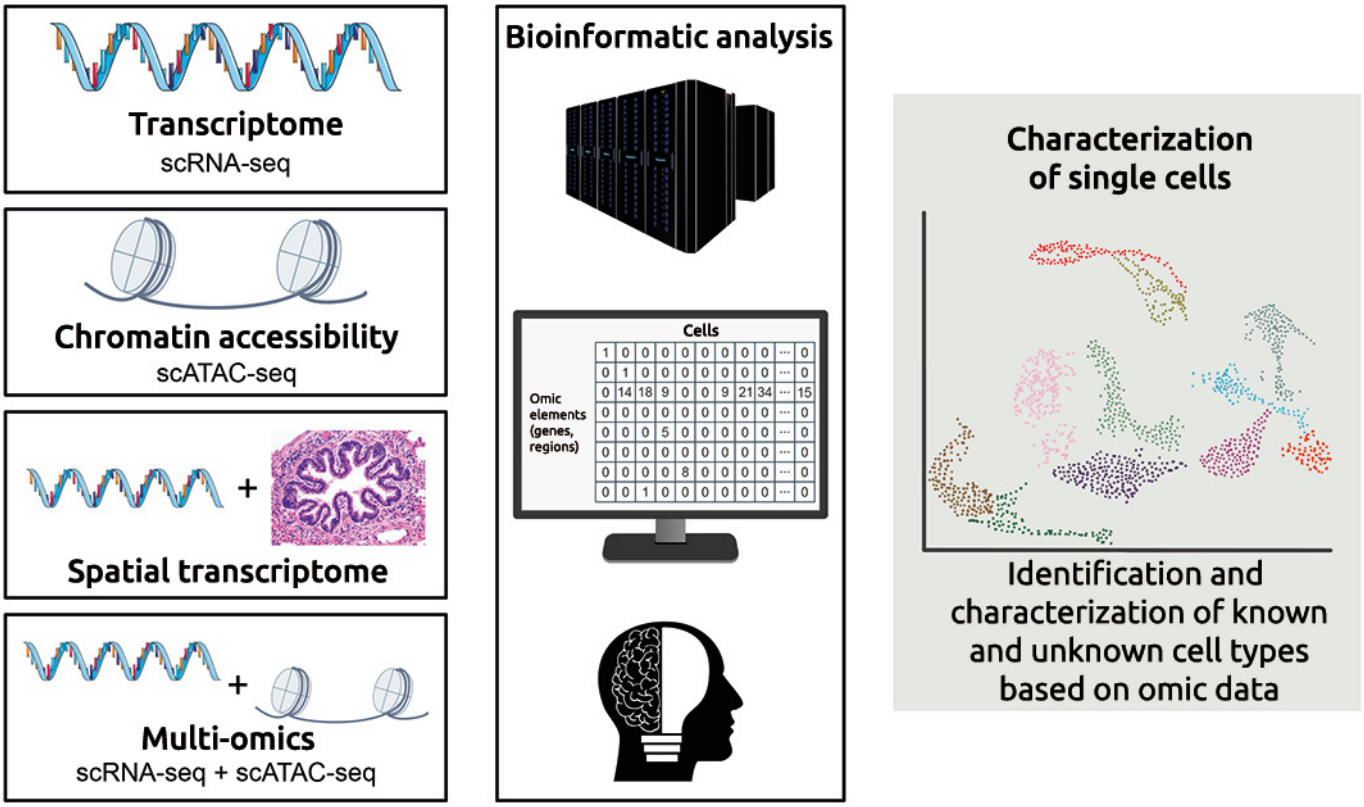

FIGURE 3. Types of omics data that can be analyzed using single cells. A complex bioinformatic analysis of these data allows the identification of the different cell populations present in a tissue.

» ries, meaning a greater number of molecules are identified per cell.

Separation via droplets. In this method, a suspension of individual cells is mixed with oil and a solution of reagents and molecular identifiers in a microfluidic device that generates tiny droplets. Each droplet contains a single cell and the necessary reagents for analysis. These droplets function as isolated compartments, enabling specific reactions to occur within each droplet without interference from other cells. This technique allows for the identification of a large number of cells in parallel (up to 10,000). It is the most popular and widely used solution today, commercially available through 10X Genomics.

Separation via molecular barcodes or indexing. This technique involves multiple rounds in which cells are distributed into various wells and tagged with molecular identifiers. Subsequently, the cells are pooled together, redistributed, and tagged again with another identifier. Each cell can then be uniquely identified based on the combination of identifiers added during each round. This method enables the analysis of a large number of cells, depending on the number of rounds performed.

OMICS DATA FROM SINGLE CELLS

Single-cell strategies were initially developed to study the transcriptome of cells. This remains the most common and widespread application of this technology, but other omics can also be analyzed using the strategies described above ***(Figure 3)***.

Transcriptome (scRNA-seq). This method allows for the identification of transcripts (RNA) present in a cell. It facilitates the classification and characterization of cells at the molecular level by revealing specific patterns of gene expression. This enables the identification of changes in gene expression that reflect the functionality and state of the cells, offering a deeper understanding of their role in biological processes and diseases.

Chromatin accessibility (scATAC-seq). In the cell nucleus, DNA is packaged »

"SINGLE-CELL" TECHNIQUES ALLOW THE STUDY OF EACH CELL IN A PANCREATIC ISLET INDIVIDUALLY

into chromatin around histones. The active regions of the genome are accessible to the enzymatic machinery responsible for transcription, while inactive regions remain packaged. Determining which chromatin regions are accessible helps identify the active parts of the genome, which are specific to each cell type. Techniques for identifying these regions rely on their increased sensitivity to breakage by enzymes known as transposases. Since there are many accessible regions per cell, the number of molecules obtained is too high to allow for sequencing all of them, meaning the percentage of fragments obtained from each cell is not very high.

Spatial single-cell (spatial single-cell). Recently, techniques have been developed to add information about the spatial localization of cells, in addition to characterizing their gene expression. This spatial single-cell technique combines the analysis of individual cells with the preservation of their original spatial location within a tissue. Using imaging technologies that capture information about the cells within their tissue context, this technique provides a detailed view of how cells interact and are distributed in the tissue. This helps in understanding cellular heterogeneity and microenvironmental interactions, which is highly relevant for studying immune attacks in T1DM.

Multi-omics (Multiome). There are technologies that allow the acquisition of two or more omics from each analyzed cell. The most common approach combines scRNA-seq and scATAC-seq. This technique is based on isolating nuclei and then characterizing the transcripts and accessible regions in each of the studied cells.

In addition to the techniques mentioned above, there are many others used to characterize various omics in individual cells, although they are not as standardized. These include the study of the proteome, methylome, histone modifications, and the three-dimensional structure of chromatin.

» SINGLE-CELL DATA ANALYSIS

The analysis of single-cell data presents significant complexity due to the use of advanced bioinformatics tools that enable the processing and analysis of large volumes of data. The vast amount of information generated requires the use of specialized computational resources, such as high-capacity clusters. This abundance of data allows the application of artificial intelligence and machine learning techniques to identify patterns and make predictions. However, the main challenge lies in interpreting this data, as more information is generated than we are currently able to analyze and comprehend.

IMPACT OF SINGLE-CELL TECHNOLOGIES ON T1DM RESEARCH

Since the development of these technologies, they have been applied to the study of various pathologies, including T1DM. Given that T1DM is a multifactorial disease involving many different cell types, single-cell studies help uncover new mechanisms involved in its development.

Single-cell RNA sequencing (scRNA-seq) of peripheral blood mononuclear cells (PBMCs) from patients with T1DM has revealed dysregulation of multiple immune cell types, including CD4+ and CD8+ T cells, B cells, NK cells, and myeloid cellsalysis (4) has characterized differential activity of transcriptional regulators in specific subsets of immune cells, providing insights into the molecular mechanisms of immune dysfunction in T1DM.

Although the immune system is primarily responsible for the destruction of beta cells in T1D, the beta cells and other pancreatic cell types also play a key role in the development of the disease (5,6). Specifically, the islets of Langerhans, contains many distinct cell types, single-cell technologies are crucial for characterizing them. Recently, a study analyzed around 80,000 pancreatic islet cells using scRNA-seq and millions of cells using flow cytometry and imaging techniques, revealing that a subset of exocrine ductal cells acquires a profile similar to tolerogenic dendritic cells in T1DM donors (7). Additionally, single-cell analysis has enabled the use of machine learning models to predict the progression of T1DM (8) providing opportunities for early interventions.

In conclusion, these findings not only deepen our understanding of the pathogenic mechanisms of T1DM but also have the potential to transform prediction, prevention, and treatment strategies for this complex disease. The identification of specific cellular states and epigenetic changes offers new opportunities to develop targeted therapies that can complement other treatments, improving clinical outcomes for T1DM patients. D

CONCLUSIONS

New single-cell techniques have revolutionized the study of T1DM genomics. These methodologies are powerful, as they allow for the characterization of cellular heterogeneity with unprecedented resolution. However, they have limitations, such as their high cost and the need for advanced bioinformatics infrastructure. Additionally, specialized knowledge is required for the correct interpretation of results. Despite these challenges, these advances enhance our understanding of T1DM pathogenesis and open new avenues for developing personalized therapies and more effective prevention strategies.

https://x.com/mireia_bioinfo
https://x.com/lorenzpasquali
https://x.com/Pasquali_Lab

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