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Single cell Transcriptomics: Mapping gene activity for better health

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Abstract

Technologies for single cell transcriptomics offer enormous promise to improve our knowledge of biology and illness. Single-cell transcriptomics has undergone a revolution in the last few years.

Introduction

Precursor techniques like single-cell qPCR, which were initially used on a limited number of genes by multiple labs in the early 1990s, are connected to the history of single-cell transcriptomics (Eberwine J et al. 1992 and Lambolez B et al 1992). Tang et al.'s 2009 (Tang F et al 2009) research of a few mouse primordial germ cells is the first instance of true single-cell transcriptomics. Although the sparsity of extremely rare cells that emerge throughout mouse development served as the inspiration for this work, the scaling of technologies to profile a large number of cells in parallel has been crucial in advancing single-cell transcriptomics. A "Moore's Law" of single-cell genomics has been nearly followed by the capacity to conduct objective single-cell transcriptome-wide analysis combined with exponential scaling in the number of cells that can be analyzed concurrently. Other single-cell transcriptomics protocols, such as tag sequencing techniques like STRT-seq, CEL-seq, MARS-seq, and full length techniques like SMART-seq/SMART-seq2, were developed after Tang et al. These procedures varied in terms of transcript coverage, amplification technique, and the degree to which liquid handling in plates was automated. Tens of thousands of cells may now be sequenced in parallel thanks to the advent of nanodroplets, picowell technology, and in situ barcoding. (Svensson et al.

2018). Recent years have seen a greater degree of dissection of the intricate regulatory and cell-cell communication networks that control cell identity because to multi-modal single-cell techniques that measure and integrate genomics readouts from various molecules (RNA, DNA, and protein) (Efremova and Teichmann 2020). Alongside the initial single-cell transcriptomics data sets, computational techniques for low-level processing, quality control, and eventually complex data interpretation were quickly created. (Stegle et al. 2015). These techniques are essential for discovering developmental trajectories that link cells to one another, identifying marker genes, and characterizing cell states and kinds from data. New computational techniques are being sparked by the more current multi-omics techniques as well as the spatial techniques (Efremova and Teichmann 2020)

A B C D E F G H

Figure 1: Single-cell technology development. Single-cell transcriptomics has made it possible to profile many cells in parallel because to significant technological advancements. Only a small number of cells could be subjected to single-cell transcriptome analysis using early manual techniques. The number of cells expanded to several thousand with the development of integrated fluidic circuits and the use of liquid handling robots. Nanodroplet and picowell technologies significantly enhanced the number of cells. With the current advancements in spatial approaches combining the spatial location of transcriptome information within tissue sections, in situ barcoding has further enhanced throughput to hundreds of thousands of cells.(A: Manual, B: Multiplexing, C: Integrated fluid circuits, D: Liquid handling robotics, E: Nanodroplets, F: Picowells, G: In situ barcoding, H: Spatial transcriptomics methods)

Techniques for characterizing gene expression profiles while preserving information on the spatial context of expression, as well as whole transcriptome spatial mapping, are rapidly developing. For many years, smFISH has been used to analyze gene expression while preserving spatial information in tissue. Methodologies based on this range from low-plex, which analyzes only a few genes in simultaneously, to highly multiplexed, which interrogates hundreds or thousands of mRNA targets in parallel, such as MERFISH, in situ sequencing, and seqFISH, which have been developed in the last five years or so. The entire transcriptome can be examined in conjunction with physical location using spatial transcriptomics techniques, which were initially created by Stahl et al. The

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most current techniques are starting to achieve single-cell resolution. Data-rich cell-atlases that map the spatial location of all cellular gene activity at high resolution will be produced more frequently when single-cell transcriptomics is combined with computational and spatial techniques.

Single-cell transcriptomics is revolutionizing our knowledge of health and illness

As previously stated, single-cell transcriptomic techniques were initially used on model organisms and are still crucial in advancing our knowledge of the cells and tissues in mice and other creatures like worms and zebrafish. Two high-throughput compendia of single-cell transcriptomics-profiled mouse tissues were released in 2018. (Tabula et al. 2018 and Xiaoping et al. 2018). These compendia offer a significant biological resource by providing data across numerous tissues utilizing somewhat similar techniques. An atlas of the mouse brain was created using a modified version of smFISH (osmFISH), which focused on particular organs and provided a precise image of each cell's location inside the tissue. (Codeluppi et al. 2018).

In the end, single-cell technology can be scaled to the complete human body. Thus, spurred by this single-cell revolution, the Human Cell Atlas (HCA) global consortium was co-founded in 2016 under the direction of Aviv Regev (Broad Institute) and Sarah Teichmann, along with colleagues who were enthusiastic about the idea. Similar to "Google maps" of the human body, HCA maps cells in human tissue utilizing high-throughput technology at single-cell resolution. High-resolution transcriptome data of individual cells in their tissue context is extremely valuable and is producing basic information on every human tissue that the HCA community studies. An essential precondition for evaluating changes in aging, disease, and responsiveness to therapeutic treatment is a healthy reference atlas of human tissues and model organisms. (Figure 2)

Studies of human development have shown how cell atlasing of human tissues can provide fresh insights into fundamental biology. New cell states (such as NK and stromal cells) and a more thorough cellular and molecular understanding of the layered architecture of this tissue and its function in maternal immune tolerance of paternal antigens were revealed by the first single-cell atlas of the maternal-fetal interface in early pregnancy (first trimester placenta and decidua). (Vento-Tormo R et al. 2018). The mechanisms underlying a healthy pregnancy were uncovered by this study, which also advances our knowledge of how these could alter and lead to diseases like pre-eclampsia.

The brain, heart, liver, thymus, gut, and kidney are only a few of the basic organs and systems to which groundbreaking single-cell atlases of development have been

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expanded. For instance, examining thymic development at single-cell resolution has improved knowledge of novel T cell types and stromal and epithelial cell states. It also reveals new principles of naïve T cell V(D)J repertoire formation, showing that the human naïve T cell repertoire is biased in its receptor gene segments before pathogen exposure. (Park J E et al. 2020)

Numerous illnesses, such as cancer and auto-inflammatory diseases, have been treated by single-cell transcriptomics. An excellent illustration of this is the airways, where a single-cell comparison of healthy and asthmatic lungs revealed unique mucosal ciliated cell states and CD4+ T cell states in both health (tissue migrating CD4+ T cell) and disease (pathogenic Th2 cells). (Vieira Braga et al. 2019) This provided a foundation for the application of single-cell transcriptomics in target discovery and identified possible new therapeutic targets. The cellular makeup of the tracheal airway has been analyzed using scRNA-seq and murine lineage tracing, which have shown that the cystic fibrosis gene CFTR (Cystic fibrosis transmembrane conductance regulator) is expressed at a higher level in a rare ionocyte cell population than in numerous ciliated cells (Montoro D T et al. 2018). These kinds of discoveries are revolutionizing our knowledge of genetically based illnesses like asthma and cystic fibrosis.

In reaction to the current worldwide epidemic, single-cell transcriptomics has been used to expand our biology knowledge of COVID-19 infection. Potential viral transmission sites can be identified using single-cell expression analysis of the coronavirus receptor ACE2, viral entry associated protease TMPRSS2, and infection-associated genes in a variety of organs in both healthy humans and non-human primates. High rates of transmission may be explained by two particular cell types in the nose that appear likely to be infection sites. (Teichmann S and Regev A 2020). With more single-cell research being conducted, this offers a useful platform for examining infection, transmission, and creating practical approaches for treatment and prevention.

Outlook for the future

Single-cell transcriptomics is a fascinating field for better in vitro cellular model engineering, including organoids. It is increasingly evident that single-cell transcriptomics can be used in a wide range of competitive and medically significant fields, such as target discovery, cell-based models, cell treatments, and regenerative medicine. Single-cell RNA-seq applied to models of the dorsal forebrain demonstrates the usefulness of single-cell transcriptomics for thorough validation of organoid models by reliably recapitulating the range of cell types observed in the human cerebral cortex (Velasco S et al. 2019). A high-

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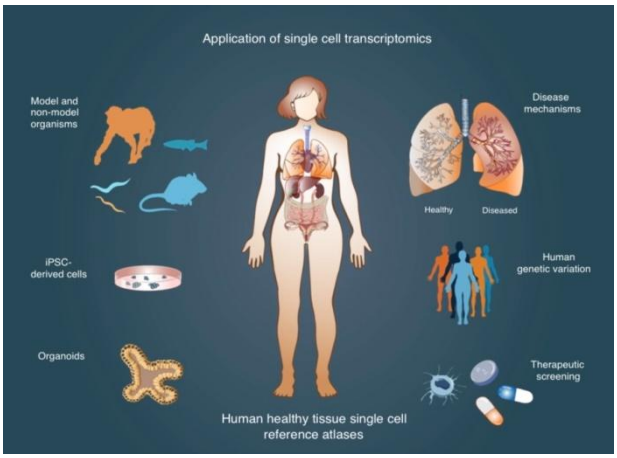
dimensional depiction of cell states, including developmental pathways starting in the early first trimester of human embryogenesis, is provided by HCA data. This gives in vitro models a precise template, and by comparing in vitro and in vivo data, we can forecast the elements needed to produce better in vitro systems like organoids and iPSC-derived cells.



Single-cell technology use (Figure 2). Reference maps of healthy human tissues, organs, and systems at single-cell resolution are being produced using single-cell transcriptomics. These methods are also being used to comprehend non-human animals, such as non-human primates and mice. Understanding disease pathways can also be achieved by analyzing genetic variation between people and healthy versus sick tissues. Single-cell responses to therapeutic screening can be measured, and factors found in in vivo single-cell transcriptome investigations can also be used to create better in vitro models.

The most exact targeting of gene editing techniques, like CRISPR-based editing, can be determined by applying insights from organ cell-atlases to the investigation of gene expression in particular cell types, as demonstrated by the accurate characterization of CFTR-expressing cells in the lung. Spatial transcriptomic methods provide a lot of potential for studying cells in tissues with complex morphology, like parts of the brain and endometrium, as they approach single-cell resolution. Understanding basic processes like development will be greatly aided by the field's efforts to address the problem of single-cell spatial transcriptomics of entire organs. These techniques are positioned as next-generation pathology and diagnostic tools due to the growing development of technology compatible with FFPE (Formalin-fixed paraffin embedde) tissue.

Combining academic research with commercial efforts to create reliable, scalable, and reasonably priced technologies has led to the explosion of success in single-cell technology. The ongoing advancement of computational techniques for data processing, analysis, and integration has served as the foundation for this.

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