Tools of the trade

Revealing genomic secrets of archival FFPE samples

Ductal carcinoma in situ (DCIS) is the most common form of early-stage breast cancer. Patients with high-grade DCIS who undergo only surgical treatment face a high recurrence rate (up to 35%). However, the genomic relationships between primary DCIS and recurrence, and the genomic events associated with progression, are poorly understood. A major technical hurdle is that most samples are preserved as formalin-fixed paraffin-embedded (FFPE) blocks, which, although ideal for histopathological analysis, creates challenges for genomic analysis due to extensive DNA damage caused by formalin fixation. To address this, we developed archival nanowell sequencing (Arc-well), a high-throughput single-cell DNA sequencing method tailored to the analysis of archival FFPE tissues. Arc-well applies optimized tagmentation-based chemistry to efficiently label and amplify the degraded DNA fragments in FFPE tissues. By integrating this chemistry with a nanowell dispensing and imaging platform, Arc-well achieves high cellular throughput (up to 2,600 cells), while substantially reducing reagent costs. Arc-well enables the processing of thousands of cells in parallel within a two-day timeframe, considerably shortening experimental time by weeks compared with the degenerate oligonucleotide-primed PCR method. Those features allow Arc-well to profile large numbers of samples at single-cell resolution.

Using Arc-well, we profiled 29,434 single cells of 27 FFPE samples from breast, lung and prostate cancers. Arc-well showed reliable technical performance in these archival samples with the median age of FFPE blocks being about 20 years (the oldest being 31 years).

Our analysis of ten paired primary DCIS (intermediate or high-grade) and recurrent breast cancer (occurring 2–16 years later) samples revealed that primary DCIS had substantial intratumoural genomic diversity, which was similar in the recurrent breast cancer samples. Clonal lineage analysis indicated that most of the primary DCIS underwent an evolutionary bottleneck, in which persistent subclones emerged years to decades later, forming the recurrent tumours. By studying the persistent subclones during recurrence, we also identified several genomic regions that harboured important breast cancer genes (for example, *PIK3CA*, *MYC*, *CCNE2* and *ZNF217*) that may associate with tumour recurrence.

Collectively, Arc-well provides a powerful, high-throughput single-cell DNA sequencing platform for analysing the vast amount of clinical FFPE samples collected worldwide. We expect that Arc-well will have broad utility for studying premalignant progression, metastatic dissemination and therapeutic response across many cancers.

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Competing interests
The author declares no competing interests.

Original article: Wang, K. et al. Archival single-cell genomics reveals persistent subclones during DCIS progression. *Cell* **186**, 3968–3982 (2023)

Tools of the trade

Investigating immune cells across time in vivo

Immune cells, patrolling through different environments of the body, are a prime example of cell plasticity in response to the environment. Circulating immune cells can alter their step when infiltrating cancer, developing excessive immune tolerance to the tumour and thus allowing cancer cells to grow unchecked. Single-cell genomics has enabled genome-wide molecular characterization of individual cells within the tumour microenvironment (TME) in an unbiased manner. However, the sequence of state transitions and thus the molecular signals that drive adaptation previously remained unknown.

Computational algorithms, such as RNA velocity and pseudotime, use arbitrary approaches to order cells based on their gene expression profiles, resulting in divergent predictions. How realistic these predictions are is impossible to tell without a ground truth of which cell state follows which. To address this gap and enable the ordering of cell states into empirical temporal trajectories to reveal time-dependent molecular changes, we developed Zman-seq.

Zman-seq provides the much-needed ground truth by intravenously injecting pulses of various fluorescent antibodies that label circulating immune cells in vivo. This behaves as a 'time stamp', as labelling pulses only label circulating immune cells and thus tissue-infiltrating cells will not be labelled by the next pulse. After a series of pulses, the fluorescent profile of each cell is measured with flow cytometry as they are sorted for single-cell RNA sequencing. Next, the fluorescent profile of each cell is overlayed on the transcriptomic single-cell atlas. This way, Zman-seq atlases reveal temporal gradients and highlight trajectories of cell-state transitions.

Using Zman-seq in a mouse model of glioblastoma, we were able to track the rapid loss of natural killer cell cytotoxic activity and found that it was driven by transforming growth factor-β (TGFβ) signalling. Moreover, Zman-seq can also be used to demonstrate the therapeutic mechanism of action. With it, we showed how an experimental, therapeutic anti-TREM2 antibody redirects the trajectory of monocytes to pro-inflammatory tumour-associated macrophages, instead of pro-tumorigenic cells.

> "captures the dynamic changes in otherwise static single-cell RNA sequencing data"

Zman-seq, via empirical time measurements, captures the dynamic changes in otherwise static single-cell RNA sequencing data. It highlights relevant cell-state transitions in vivo and lays the foundations for dynamic TME network models, paving the way for novel TME models and immunotherapy strategies.

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Competing interests
D.K. is a part of a pending patent related to the Zman-seq technology.

Original article: D. K. et al. Time-resolved single-cell transcriptomics defines immune trajectories in glioblastoma. *Cell* **187**, 149–165.e23 (2024)