DEVELOPMENT

Transcriptomics of developmental fate

“the value (and limitations) of scRNA-seq for predicting developmental fate”

Single-cell RNA sequencing (scRNA-seq) has enabled the phenotypic profiling of individual cells at unprecedented resolution. However, as cells are inherently destroyed during scRNA-seq procedures, identifying temporal aspects of profiled cells is a challenge. Two new studies combine scRNA-seq with either lineage tracing or a computational algorithm to link transcriptomes to future developmental trajectories.

In their study, Weinreb, Rodriguez-Fraticelli et al. focused on the mouse haematopoietic system. To enable a dynamic assessment of both lineage and transcriptional state, they delivered a DNA barcode library to haematopoietic stem and progenitor cells (HSPCs). As the barcode is contained in the 3' untranslated region of an expressed *GFP* gene, scRNA-seq analyses can read out both the current transcriptional state and the lineage barcode. First allowing the cells to divide for 2 days generated transcriptionally equivalent 'sister' cells for each barcode. This approach allowed serial sampling of clones: half the cells were captured at this early time point, and the remainder were sampled at day 4 and 6 of differentiation in culture, or week 1 and 2 post-transplantation in recipient mice. The authors termed their approach LARRY (lineage

and RNA recovery), and they note that it may be applied to diverse biological systems.

The classic branching model of haematopoietic differentiation involves mature cell types being reached through defined intermediate cell types of progressively narrower differentiation potential. Single-cell studies recently clarified that progenitors for different mature cell types do not partition into discrete states. Instead, they form a structured continuum. Using their barcoding approach, the team could parse out differences in lineage commitment in otherwise similar cells.

The key question that the authors addressed is whether scRNA-seq was able to fully parse out the steps in differentiation. There is a common hope that this will be the case across many tissues. To address this question they compared the behaviour of sister cells using a 'twin study' design borrowed from classical genetics to quantify the heritability of traits. The authors found that lineage outcomes were strongly heritable. However, only part of this heritability could be explained by the scRNA-seq profile of the cells. Therefore, the results indicate that transcriptomes alone may not determine the future cell fate of HSPCs. Hence, these results argue for looking beyond scRNA-seq. The molecular nature of any additional fate determinants — whether based on chromatin state, protein levels, cell organization or other factors — remains to be characterized.

In a separate study, Gulati, Sikandar et al. investigated whether any particular transcriptional signatures could be broadly predictive of developmental potential ('stemness') across various species, cell types and scRNA-seq platforms. They trained their model on diverse types of

existing scRNA-seq data sets with known differentiation statuses, such as human embryonic stem cells sampled during in vitro differentiation, or in vivo development for mice and *Caenorhabditis elegans*. The authors tested nearly 19,000 gene sets for those whose expression correlated with developmental potential.

Despite the complexity of the training data and models tested, a remarkably simple metric emerged as the strongest predictor of developmental potential: the number of genes expressed per cell. The authors used noise smoothing to refine this metric to form the computational framework CytoTRACE, which showed strong predictive value in independent scRNA-seq validation data sets.

Biologically, the relationship between transcriptional diversity and developmental potential might reflect the progressive compaction of chromatin regions and restriction of gene expression programmes during differentiation.

Beyond the broad applicability across systems, the authors applied CytoTRACE to breast cancer, where stemness is associated with poorer clinical outcomes. From scRNA-seq data from eight patients, CytoTRACE inferred less-differentiated cells, from which marker genes of stemness were identified. Knockdown of one of these genes, *GULP1*, inhibited growth of human breast cancer cells in culture and as xenografts in mice. Further work will be required to determine the role of *GULP1* in cancer cells versus normal cells and whether it could be a promising candidate drug target.

These studies highlight the value (and limitations) of scRNA-seq for predicting developmental fate.

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ORIGINAL ARTICLES Weinreb, C. et al. Lineage tracing on transcriptional landscapes links state to fate during differentiation. *Science* **367**, eaaw3381 (2020) | Gulati, G. S. et al. Single-cell transcriptional diversity is a hallmark of developmental potential. *Science* **367**, 405–411 (2020)

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