STEM CELLS

Multiplex fate mapping

barRNAseq allows detailed explorations of signaling pathways that control embryonic differentiation.

Embryonic stem cells (ESCs) are pluripotent stem cells harboring the potential to differentiate into all somatic cell types. Understanding the precise mechanisms that govern ESC differentiation holds great promise for the fields of regenerative medicine and developmental biology. Researchers at Harvard Medical School have developed barRNAseq, a combinatorial oligonucleotide-based single-cell RNA sequencing (scRNAseq) method to observe the effects of multiple signaling pathways on ESC differentiation.

“We wanted to have a more complete picture of what happens if you take embryonic stem cells and expose them to all of the possible differentiation factors that might contribute to each step, and eventually we could build this comprehensive map of the stem cell differentiation landscape,” says Richard Sherwood, lead investigator of this study.

To measure the contribution of specific signaling pathways to the global transcriptomic landscape, the researchers treated cells in a multiwell plate with specific combinations of activators or inhibitors. Each well received a unique treatment and was transfected with a combination of short oligonucleotides called barcodelets. Next, the cells from multiple wells were pooled and analyzed by scRNAseq, in which each cell-specific barcodelet can be associated with its treatment combination. The team then developed a statistical analysis framework modeling the top 1,000 most variable genes as if they arose from a normal distribution.

One of the key considerations in the application of barRNAseq is the experimental design, in which the power to multiplex can come at the cost of resolution. “There is definitely a tradeoff between the number of conditions you can have in a single experiment and the resolution at which you can do analysis. I think in terms of using this for your systems, you have to keep in mind what the goals of your analysis are when you’re doing your planning. I think [the] experimental design is very important,” explains first author Grace Hui Ting Yeo.

Using this method, the researchers tested the effects of five key signaling pathways on mouse ESC germ layer specification. One of the landmark stages of embryogenesis is the development of three germ layers (ectoderm, mesoderm and endoderm) from the primitive epiblast stage. barRNAseq not only revealed the complex interactions of these pathways on ESC differentiation but could also reveal previously unidentified regulatory molecules necessary for germ layer development. For instance, they showed that the expression of the gene *Lefty1*, a target of Tgf- β , is greatly enhanced in the presence of retinoic acid and in the absence of Bmp. *Lefty1* is an important gene for the development of the left–right axis.

Next, the researchers tested the potential of barRNAseq to identify gene regulation on a cell fate level. By training a classifier on published cell-atlas-derived datasets, they could identify the signatures of specific embryonic subpopulations using a supervised approach. Using a different method, unsupervised clustering, they were able to identify paths to cell fates that had previously not been described. They could, for example, identify a subpopulation of cells expressing notochord-related genes that were highly enriched in Tgf- β -containing cultures in the absence of retinoic acid, Wnt or Bmp signaling.

barRNAseq is a powerful method that can help unravel the intricate signaling networks that control ESC differentiation into various cell fates. The team will continue to exploit this technology by delving deeper into additional pathways that may regulate embryonic cells. “I think it’ll be exciting in the future to just focus on more unexplored territory where the power of this technology can really be maximized,” says Sherwood.

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Research paper

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