BIOLOGICAL TECHNIQUES

Getting to know the neighbors

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To understand a cell's function or niche requires knowledge of how it interacts with other cells, both proximally and at a distance. For many tissues, this basic information about cell-cell interactions is lacking.

A new study from Nature Methods begins to address this shortcoming. In this report, members of Alexander van Oudenaarden's lab at the Hubrecht Institute in the Netherlands present a technique called ProximID to identify cellular associations, which they demonstrate with two different tissues.

Beginning with mouse bone marrow, the investigators dissociated tissue into small interacting structures, which were then microdissected to individual cells and subjected to single-cell mRNA sequencing. Using the respective transcriptomic signature of separated cells, they characterized cell types present in the small interacting structures. By then comparing observed associations to those predicted by chance, they could determine cell-cell interactions in the structures and by extension the tissue.

After applying this technique to bone marrow, the authors identified the previously characterized macrophage/erythroblast connection, a.k.a. the erythroblastic island. As a demonstration of the technique's utility, they also described previously unknown cell-cell interactions, including an association between megakaryocytes and neutrophils. The investigators also observed a connection between myeloblasts/promyelocytes and plasma cells. Both of these novel interactions were validated using an orthogonal technique, single-molecule fluorescence in situ hybridization (sm-FISH).

According to Jean-Charles Boisset, first author and a postdoc in the lab, they were excited with these novel findings. However, while other scientists thought the bone marrow results were interesting, the method received some criticism regarding its lack of throughput. The group took this as a challenge.

To address the speed problem in their next round of experiments, the authors modified their techniques. Working with crypt tissue derived from small intestine of mice, they gently dissociated tissue to structures consisting of doublets and triplets. The structures' mRNA was then sequenced, instead of microdissecting and sequencing those cells. As a new step, investigators partitioned crypt tissue into individual cells and separated them by flow cytometry prior to analyzing each cell's transcriptome. Using the sequencing data from the flow cytometry-separated cells, the investigators trained machine learning algorithms to recognize interactions amongst the sequenced structures.

From the small intestine experiments, authors characterized a previously known Paneth cell Lgr5$^+$ stem cell interaction. Additionally, they identified a new association between Lgr5$^+$ stem cells and Tac1$^+$ enteroendocrine cells, which was verified using sm-FISH.

Boisset estimated his experimental throughput was increased about five-fold using the latter technique. However, he did qualify this by pointing out that the adaptive algorithms misidentified some interactions, so there is a tradeoff between thoroughness and speed. Nonetheless, he believes the method could be particularly useful in defining stem cell niches and characterizing cancer cell interactions.

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