STEM CELLS

Reprogrammed cells leave their past lives behind

Genetic background trumps tissue of origin as a source of variability between induced pluripotent stem cell lines, diminishing the role of somatic memory in reprogrammed cells.

Ever since researchers started bestowing pluripotency on differentiated cells, there has been a question of whether the newly born stem cells retain a lingering epigenetic memory of their former selves. Early studies showed that induced pluripotent stem cell (iPSC) lines are not all equal and differ in qualities such as gene expression, a fact that was ascribed to tissue-of-origin effects. The issue may have important practical consequences: a stem cell with a memory of muscle might be less inclined to differentiate into a neuron than back to muscle.

But recent work downplays the importance of memory. For example, researchers at the Wellcome Trust Sanger Institute in Cambridge, UK, showed that gene expression in iPSCs generated from three different cell types varied according to the individual, and less so by the tissue, from which they were derived. Two new studies, including research from the laboratory of Yoav Gilad at the University of Chicago, now confirm and extend the conclusion that somatic memory has a limited role in reprogrammed cells.

The Gilad lab studies gene regulation. Much of their work has used immortalized lymphoblastoid cell lines, but they are moving to iPSCs because with those cells it is possible to examine tissue-specific gene regulation and, unlike with primary cells, to do it from the same individuals repeatedly, enabling replication and perturbation experiments. And as Nick Banovich, a graduate student in the lab, points out, "if your equipment breaks or someone drops the tube, your experiment is not lost." Large tissue-expression panels such as from the Genotype-Tissue Expression (GTEx) project have been extremely useful but are based on a finite source of postmortem tissue.

Banovich and co–first author Courtney Burrows embarked on the somatic-memory work as a form of quality control; they wanted to ensure that an analysis of gene regulation across individuals would be meaningful. The team of researchers applied episomal reprogramming to three lymphoblast lines and one fibroblast line from each of four individuals, then extracted DNA and RNA from the primary cells and their derived stem cell lines (Burrows *et al.*, 2016). Using gene expression and cytosine methylation arrays, they found that the proportion of variance explained by the donor individual was around 16% for the both data types, compared to only around 7% for tissue of origin. Moreover, they could detect only a single differentially expressed gene between fibroblast- and lymphoblast-derived lines.

"Our paper doesn't disprove that there could be some other mechanism—some other epigenetic regulator that is carrying this memory over," says Banovich, but he notes that so far only DNA methylation has been implicated in somatic memory. He also believes that the results will be true across different cell types of origin.

Indeed, similar work led by Timo Otonkoski and Ras Trokovic at the University of Helsinki on iPSC lines derived from fibroblasts and peripheral blood mononuclear cells shows that differences in gene expression, DNA methylation and differentiation potential are largely driven by genetic differences in the donor, rather than the tissue type (Kyttälä *et al.*, 2016). Their work also establishes the feasibility of generating large iPSC panels for biobanks from these readily available tissues, without fearing that tissue source will confound analysis.

The key to the new insight on memory is in the scope of the comparison. "What's interesting is that some of the earlier papers actually see a similar proportion of epigenetic memory to what we see," says Banovich. But benchmarking against variation in individuals puts this variation in perspective.

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RESEARCH PAPERS

Burrows, C.K. *et al.* Genetic variation, not cell type of origin, underlies the majority of identifiable regulatory differences in iPSCs. *PLoS Genet.* **12**, e1005793 (2016).

Kyttälä, A. *et al.* Genetic variability overrides the impact of parental cell type and determines iPSC differentiation potential. *Stem Cell Reports* **6**, 200–212 (2016).