STEM CELLS

Testing pluripotency

Reference datasets of gene expression and DNA methylation in human pluripotent stem cell lines are reported.

Unlike pluripotent stem cells from an experimental animal, human stem cells—both embryonic stem cells (ESCs) and induced pluripotent stem (iPSCs)—cannot be rigorously functionally tested *in vivo*. As researchers in more and more laboratories generate human iPSC lines, there is a need for simple, robust and biologically meaningful methods to assess the potential of a given human stem cell line.

Two studies proposing such methods have been published recently (Bock *et al.*, 2011 and Müller *et al.*, 2011), both making use of the increasing ease with which a cell can be globally profiled at the genomic or transcriptomic level. If one generates a reference map of pluripotency using known human pluripotent stem cells, the thinking goes, any newly derived or reprogrammed line can then be compared to this reference, and its properties and most likely applications can be assessed.

Alexander Meissner, Kevin Eggan and colleagues at Harvard University generated their reference dataset from 20 widely used human ESC lines. They used reduced representation bisulfite sequencing to profile DNA methylation at three-quarters of all gene promoters and most CpG islands in the genome of these cell lines, and Affymetrix microarrays to determine transcript levels for all genes. Taken together, these data represent what the researchers call a 'reference corridor', a range of DNA methylation and gene expression values that can be considered normal for a human pluripotent stem cell.

Reference in hand, the researchers then probed how well other cell lines conform. Comparing DNA methylation and gene expression in 12 human iPSC lines to the reference value for each gene, they obtained a 'deviation scorecard' for each human iPSC line. Notably, especially in light of the recent flurry of comparative studies of ESC and iPSC genomes, most iPSCs tested in this study fell within the range of normal variation, though the average deviation from the reference was higher for human iPSC than for human ESC lines.

Following similar principles, Franz-Josef Müller at the Zentrum für Integrative Psychiatrie in Kiel and Jeanne Loring at the Scripps Institute generated gene expression datasets from hundreds of known human pluripotent and nonpluripotent cell samples. They then used this information to train bioinformatic classifiers that report whether a test cell line is likely to be pluripotent, and if so, whether it is also normal. The goal of their open-access tool, PluriTest, is to provide other researchers with a way to assess a cell line of interest, using microarray data that are relatively simple to generate in any lab.

Finally, Meissner, Eggan and colleagues introduce a 'lineage scorecard' to assess the differentiation proclivities of a given cell line. To do this, they put pluripotent stem cell lines through an embryoid body–based differentiation protocol and measured the expression levels of 500 selected marker genes for the three germ layers. The patterns of these markers should be indicative of the differentiation preferences of a given cell line. The researchers found that their predictions for neural differentiation in several human iPSC lines coincided with experimental findings from Hynek Wichterle and colleagues at Columbia University, who conducted a parallel directed differentiation study on the same lines (Boulting *et al.*, 2011).

Inherent to the promise of human pluripotent stem cell lines, either as research or therapeutic tools, is their ability to generate many functional human cell types. In the absence of perfect *in vivo* assays for pluripotency, the most productive assessment of these cells is likely to be pragmatic. Is a given cell line useful? Is it normal? What are its differentiation tendencies? The move toward systematic comparisons of both global molecular properties and function of human pluripotent stem cell lines is promising and will hopefully lead to a more complete picture not only of what a pluripotent cell is but of which deviations from this state matter and which do not.

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RESEARCH PAPERS

Bock, C. *et al.* Reference maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. *Cell* **144**, 439–452 (2011).

Boulting, G.L. *et al.* A functionally characterized test set of human induced pluripotent stem cells. *Nat. Biotechnol.* **29**, 279–286 (2011).

Müller, F.-J. *et al.* A bioinformatic assay for pluripotency in human cells. *Nat. Methods* **8**, 315–317 (2011).