New insights into cellular reprogramming revealed by genomic analysis

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The ability to drive somatic, or fully differentiated, human cells back to a pluripotent or “stem cell” state would overcome many of the significant scientific and social challenges to the use of embryo-derived stem cells and help realize the promise of regenerative medicine.

Recent research with mouse and human cells has demonstrated that such a transformation (“reprogramming”) is possible, although the current process is inefficient and, when it does work, poorly understood. But now, thanks to the application of powerful new integrative genomic tools, a cross-disciplinary research team from Harvard University, Whitehead Institute, and the Broad Institute of MIT and Harvard has uncovered significant new information about the molecular changes that underlie the direct reprogramming process. Their findings are published online in the journal *Nature*.

“We used a genomic approach to identify key obstacles to the reprogramming process and to understand why most cells fail to reprogram,” said Alexander Meissner, assistant professor at Harvard University’s Department of Stem Cell and Regenerative Biology and associate member of the Broad Institute, who led the multi-institutional effort. “Currently, reprogramming requires infecting somatic cells with engineered viruses. This approach may be unsuitable for generating stem cells that can be used in regenerative medicine. Our work provides critical insights that might ultimately lead to a more refined approach.”

Previous work had demonstrated that four transcription factors —

proteins that mediate whether their target genes are turned on or off — could drive fully differentiated cells, such as skin or blood cells, into a stem cell-like state, known as induced pluripotent stem (iPS) cells. Building off of this knowledge, the researchers examined both successfully and unsuccessfully reprogrammed cells to better understand the complex process.

“Interestingly, the response of most cells appears to be activation of normal ‘fail safe’ mechanisms”, said Tarjei Mikkelsen, a graduate student at the Broad Institute and first author of the Nature paper. ”Improving the low efficiency of the reprogramming process will require circumventing these mechanisms without disabling them permanently.”

The researchers used next-generation sequencing technologies to generate genome-wide maps of epigenetic modifications — which control how DNA is packaged and accessed within cells — and integrated this approach with gene expression profiling to monitor how cells change during the reprogramming process. Their key findings include:

1. Fully reprogrammed cells, or iPS cells, demonstrate gene expression and epigenetic modifications that are strikingly similar, although not necessarily identical, to embryonic stem cells.

2. Cells that escape their initial fail-safe mechanisms can still become ‘stuck’ in partially reprogrammed states.

3. By identifying characteristic differences in the epigenetic maps and expression profiles of these partially reprogrammed cells, the researchers designed treatments using chemicals or RNA interference (RNAi) that were sufficient to drive them to a fully reprogrammed state.

4. One of these treatments, involving the chemotherapeutic

5-azacytidine, could improve the overall efficiency of the reprogramming process by several hundred percent.

“A key advance facilitating this work was the isolation of partially reprogrammed cells,” said co-author Jacob Hanna, a postdoctoral fellow at the Whitehead Institute, who recently led two other independent reprogramming studies. “We expect that further characterization of partially programmed cells, along with the discovery and use of other small molecules, will make cellular reprogramming even more efficient and eventually safe for use in regenerative medicine.”

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