New single-cell analysis reveals complex variations in stem cells

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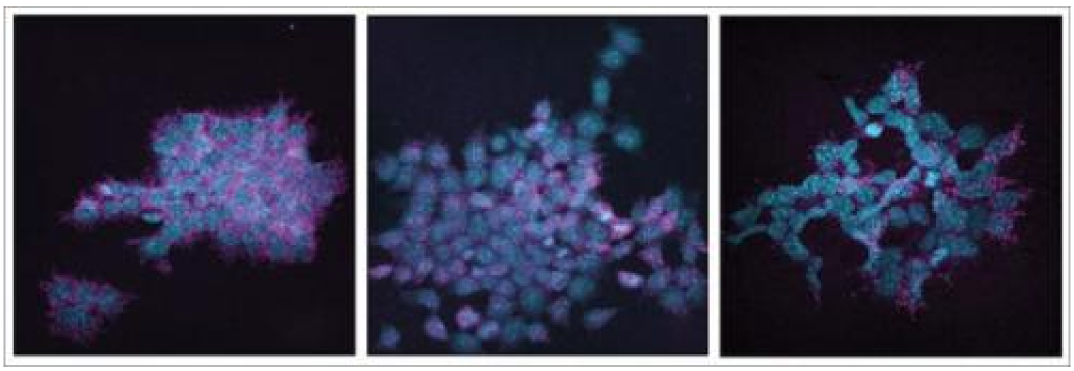

Researchers discovered many small nuances in pluripotency states of stem cells by subjecting the cells to various perturbations and then analyzing each individual cell to observe all the different reactions to developmental cues within a stem cell colony. Credit: Harvard's Wyss Institute

Stem cells offer great potential in biomedical engineering due to their pluripotency, which is the ability to multiply indefinitely and also to differentiate and develop into any kind of the hundreds of different cells and bodily tissues. But the precise complexity of how stem cell development is regulated throughout states of cellular change has been difficult to pinpoint until now.

By using powerful new single-cell genetic profiling techniques, scientists at the Wyss Institute for Biologically Inspired Engineering and Boston Children's Hospital have uncovered far more variation in pluripotent stem cells than was previously appreciated. The findings, reported today in *Nature*, bring researchers closer to a day when many different kinds of stem cells could be leveraged for disease therapy and regenerative treatments.

"Stem cell colonies contain much variability between individual cells. This has been considered somewhat problematic for developing predictive approaches in stem cell engineering," said the study's co-senior author James Collins, Ph.D., who is a Wyss Institute Core Faculty member, the Henri Termeer Professor of Medical Engineering & Science at MIT, and a Professor of Biological Engineering at MIT. "Now, we have discovered that what was previously considered problematic variability could actually be beneficial to our ability to precisely control stem cells."

The research team has learned that there are many small fluctuations in the state of a stem cell's pluripotency that can influence which developmental path it will follow.

It's a very fundamental study but it highlights the wide range of states of pluripotency," said George Daley, study co-senior author, Director of Stem Cell Transplantation at Boston Children's Hospital and a Professor of Biological Chemistry and Molecular Pharmacology at Harvard Medical School. "We've captured a detailed molecular profile of the different states of stem cells."

Taking this into account, researchers are now better equipped to manipulate and precisely control which cell and tissue types will develop from an individual pluripotent stem cell or stem cell colony.

"The study was made possible through the use of novel technologies for studying individual cells, which were developed in part by collaborating groups at the Broad Institute, giving our team an unprecedented view of stem cell heterogeneity at the individual cell level," said Patrick Cahan, co-lead author on the study and Postdoctoral Fellow at Boston Children's Hospital and Harvard Medical School.

Researchers explored the developmental landscape of pluripotent stem cells by perturbing them with variants such as different chemicals, culture environments, and genetic knockouts. Then, they analyzed the individual genetic makeup of each cell to observe micro-fluctuations in each stem cell's state of pluripotency. They discovered many small nuances in the way stem cells are influenced by internal, chemical and environmental cues, revealing a complex "decision making" circuit of developmental regulators.

"These emerging single-cell analytics allow us to classify cells very precisely and identify regulatory circuits that control cell states," said the study's co-lead author Roshan Kumar, a former Wyss Institute Postdoctoral Fellow who is now a Senior Scientist at HiFiBiO Inc. and a Visiting Scholar at the Wyss Institute. "The real motivating force behind this study was to understand the causes and consequences of differences between individual stem cells and how the balance of key regulators within a cell can affect that cell's developmental outcome."

Looking at the findings, the researchers now believe there is a "code" that relates patterns of dynamic behavior in stem cell regulatory circuits to the developmental path a cell ends up taking. By leveraging that code, they hope to dial in precisely to specific individual cell states and to use them for a variety of purposes, such as creating certain cell types that a patient's body may be unable to produce on its own.

"The ability to understand and program stem cells throughout changing states of pluripotency is a critical necessity for the success of regenerative medicine," said Wyss Institute Founding Director Donald Ingber, who is also Judah Folkman Professor of Vascular Biology at

Harvard Medical School and Boston Children's Hospital, and Professor of Bioengineering at the Harvard School of Engineering and Applied Sciences. "By making stem cell engineering more predictive, we hope to leverage the versatility of controllable pluripotent stem cells to address a wide range of diseases and injuries."

More information: Deconstructing transcriptional heterogeneity in pluripotent stem cells, *Nature* 516, 56–61 (04 December 2014) DOI: 10.1038/nature13920

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