

Embryonic stem cell strategy advanced with new finding

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UCSF scientists are reporting what they say is a significant improvement in the technique for genetically reprogramming mouse cells to their embryonic state, a process that transforms the cells, in essence, into embryonic stem cells.

The finding, published on-line as an immediate early publication in *Cell Stem Cell* (Sept. 6, 2007), builds on the strategic breakthrough reported by Shinya Yamanaka, MD, PhD, in 2006, and confirmed in the spring of 2007 both by Yamanaka's team and, in independent studies, by scientists at MIT, Harvard and UCLA.

The advance by the UCSF team should accelerate research aimed at improving the original strategy, the team says, and increase its potential use for studying disease development and creating patient-specific stem-cell based therapies.

The work is the result of a collaboration between the labs of Miguel Ramalho-Santos, PhD, and Robert Blelloch, MD, PhD, of the UCSF Institute for Regeneration Medicine.

“The new technique removes a major technical hurdle that has likely discouraged many labs around the world from carrying out studies on the strategy,” says senior author Ramalho-Santos, a UCSF Fellow and a member of the Diabetes Center. For separate reasons, he says, removal of the hurdle increases the technique's potential use in developing patient-specific cellular therapies.

“Now, laboratories will be able to use the approach to study a broad range of normal and diseased cells of interest,” says the first author of the study, Blelloch, an assistant professor of urology. “There will be a much greater ability to precisely dissect the mechanisms of reprogramming and to identify the genes that will be most effective in transforming adult cells.”

Yamanaka’s strategy -- over-expressing certain genes in mouse skin cells to initiate reprogramming – relied on the insertion of a foreign “drug resistance” gene into the mouse skin cells. This gene would “switch on” in those cells that successfully converted to embryonic stem cells, thus providing a means of detecting them. The drawbacks of this technique were that it was technically difficult to carry out and, because it involves a foreign gene, would raise safety concerns that would hinder its use in cell-based therapies.

In the current study, the UCSF scientists developed an alternative to this genetically engineered “switch” technique. They developed serum-free conditions in the cell culture dish that both promoted more successful reprogramming and generated embryonic stem cells that could be detected based on their form and structure, alone.

Scientists are interested in reprogramming because of its potential for developing human embryonic stem cells that contain the genetic makeup of individual patients. In theory, any patient’s cell, say, a skin cell, could be reprogrammed. If the resulting embryonic stem cell could then be prompted in the culture dish to specialize into one of the various cell types of the body, such as of the heart, lung and brain, the resulting cells could provide the starting point for a host of clinical-research strategies.

Researchers could create dopamine-producing cells from Parkinson’s disease patients and study them in the culture dish to learn the earliest steps of disease development. They could also test experimental drugs on

such cells in the culture dish.

Alternatively, they could generate healthy specialized cells from patients who had donated their genetic material, and transplant them into tissues -- without the risk of prompting immune rejection -- to treat failing hearts, neurological diseases such as Parkinson's disease and amyotrophic lateral sclerosis, spinal cord injury and diabetes.

The reprogramming strategy pioneered by Yamanaka -- who in August began his transition from Kyoto University to the UCSF-affiliated Gladstone Institute of Cardiovascular Disease and UCSF -- involved over-activating four genes in mouse skin cells in the culture dish. His team showed that over-expressing these genes -- oct4, sox2, klf4 and c-myc -- can cause the full complement of genes in mouse cells to lose their adult functions and begin functioning as they would have as embryonic stem cells. Yamanaka named these cells "induced pluripotent (iPS) cells."

But because only a very low percentage of cells complete reversion to the embryonic stem cell state with this technique, and because the cells are situated among millions of cells in the culture dish that do not complete the transformation, the scientists had a difficult time identifying the fully reprogrammed cells. Thus, they developed the technique of inserting the foreign "drug-resistance" gene into the mouse skin cells. This gene was designed to only "switch on" in cells that completed the reversion to the embryonic stem cell state. With addition of the drug to the culture dish, the vast majority of cells, those that had not reverted to embryonic stem cells, died. Only those that had reverted survived and could then be expanded.

With the alternative technique developed by the UCSF team, the efficiency of embryonic stem cell production remained low. However, the mouse skin cells that did start to revert to embryonic stem cells could

readily be identified by their form and structure in the absence of any drug. The researchers went on to show that these cells indeed behaved like embryonic stem cells and could give rise to all cell types of the body.

Separately, the team demonstrated that reprogramming could be achieved when one of the four genes over-expressed to initiate reprogramming -- c-myc -- was replaced with a related gene, known as n-myc. These genes are involved in the formation of different tumors, so by beginning to replace genes in this method the researchers may find combinations of reprogramming genes that are safer, says Blelloch.

“Studies should address the relative efficacy of n-myc versus c-myc in reprogramming and whether n-myc reactivation, like c-myc, results in tumor formation,” he says.

An ongoing limitation of the Yamanaka method, notes Blelloch, is that it requires viral-mediated integration of four foreign genes – so-called transgenes. The goal would be to add the genes only temporarily, or to use chemical compounds that could mimic the effect of the genes in the cells. This will be a key focus of ongoing studies, he says.

The biggest hurdle, of course, says Ramalho-Santos, will be translating the methods from the mouse to human cells, a process that could take years. Researchers around the world, including Ramalho-Santos, Blelloch and Yamanaka, are working intently on this challenge.

“It’s a very exciting time in stem cell biology, as exemplified in the studies of reprogramming,” says Ramalho-Santos. “It’s fascinating enough that an embryonic stem cell can give rise to all cell types of the body. But that’s what embryonic stem cells do. They grow and in the end give rise to the whole organism.

“But taking back a differentiated cell to the embryonic stem cell state – that’s truly mesmerizing. It goes against the flow of development -- and yet we can do it. And we’re getting easier technical ways to do it.”

Source: University of California - San Francisco

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