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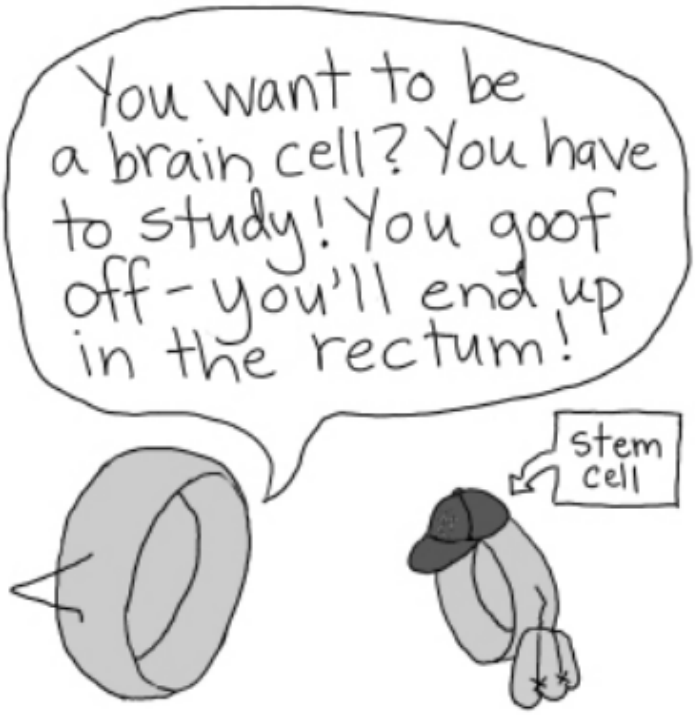
SPEAKER: CLAUDIA LOVELL

FEBRUARY 10, 2014

REPROGRAMMING & INDUCTION


You want to be a brain cell? You have to study! You goof off - you'll end up in the rectum!
stem cell

STEM-CELL INDUCTION MADE SIMPLER

Induced pluripotent stem cells made by inserting genes at just one location.

Brendan Borrell

Adult mouse cells can now be reprogrammed to a stem-cell-like state with the help of a single genetic insertion — rather than the multiple gene insertions required in the past. The advance also enables reprogrammable mice to be maintained in the lab generation after generation.

Mouse cells can be reprogrammed by a single cassette of genes. *CORBIS*

Three years ago, Shinya Yamanaka at Kyoto University and his colleagues made a splash by creating the first induced pluripotent stem (iPS) cells, which can develop into any of the body's cell types. Because they are obtained using adult body cells, iPS cells hold the potential for being used to develop human therapies without the ethical concerns associated with stem cells obtained from embryos. So far, iPS cells have been reprogrammed from a wide variety of somatic cell types, including skin, blood and liver cells, but scientists are still unsure how iPS cells compare to true embryonic stem cells.

One challenge has been the fact that to induce pluripotency, four reprogramming genes must be inserted into the genome — Oct4, Sox2, Klf4 and c-Myc. This requires the use of multiple retroviruses, meaning the genes end up in random locations in the mouse genome, which can interfere with the function of the mouse's own genes. Moreover, offspring of these mice must be screened to ensure that they contain all of the required reprogramming genes.

Now, two teams of researchers — one led by Rudolf Jaenisch at the Massachusetts Institute of Technology in Cambridge and the other led by his former student Konrad Hochedlinger at Harvard University, also in Cambridge, Massachusetts — describe a technique in Nature Methods that avoids these difficulties[1,2].

Time-saver

The researchers combined the four mouse reprogramming genes onto a piece of DNA, known as a cassette, which they inserted at a single locus in the mouse genome. The mice were then bred, and their somatic cells were transformed into iPS cells following the addition of the antibiotic doxycycline, which triggers the cassette to express the four reprogramming genes.

"The advantage of this method is that the single gene has been introduced to a defined locus," says Hochedlinger, "The problem with a virus is that you never really know where it landed in the genome or how

well it was expressed." By eliminating this variability, Hochedlinger says that the technique will eliminate the need for further screening in the mice and free up the equivalent of one full-time employee in his lab. "I'm very happy," he says.

"The problem with a virus is that you never really know where it landed in the genome."

The technique may also help to answer lingering doubts about the differences between iPS cells and embryonic stem cells. A study earlier this year in Cell Stem Cell showed that hundreds of genes are differentially expressed in the two cell types[3], and another revealed that iPS cells are not as efficient as embryonic stem cells at differentiating into all cell types[4]. Matthias Stadtfeld of Harvard University, who is first author on one of the reprogramming studies[2], says that it will now be possible to compare two genetically matched cell types and ask if iPS cells are as useful as embryonic stem cells. "We are fairly confident you can reprogram any cell type, the question is: are we ending up with the same quality of cells in the end?"

Super strains

Other experts agree that the advance will circumvent limitations with iPS technologies. "I've been hoping these guys would make these strains of mice," says stem-cell biologist George Daley of the Children's Hospital in Boston, Massachusetts, who was not involved in the research.

Although some researchers have developed non-genetic systems to reprogram cells using proteins or small molecules (see 'Stem-cell therapies closer to the clinic'), Daley points out that such methods are currently "incredibly inefficient". To improve efficiency and safety so that these techniques can be used in humans, scientists could potentially create lines of mice with just three of the four reprogramming genes, and screen for chemicals that could be used as an alternative to inserting the fourth reprogramming gene.

"Fundamentally, everyone is looking to improve the efficiency of reprogramming using chemicals, proteins and the like," Daley says. "These two papers give you a substrate on which to work."

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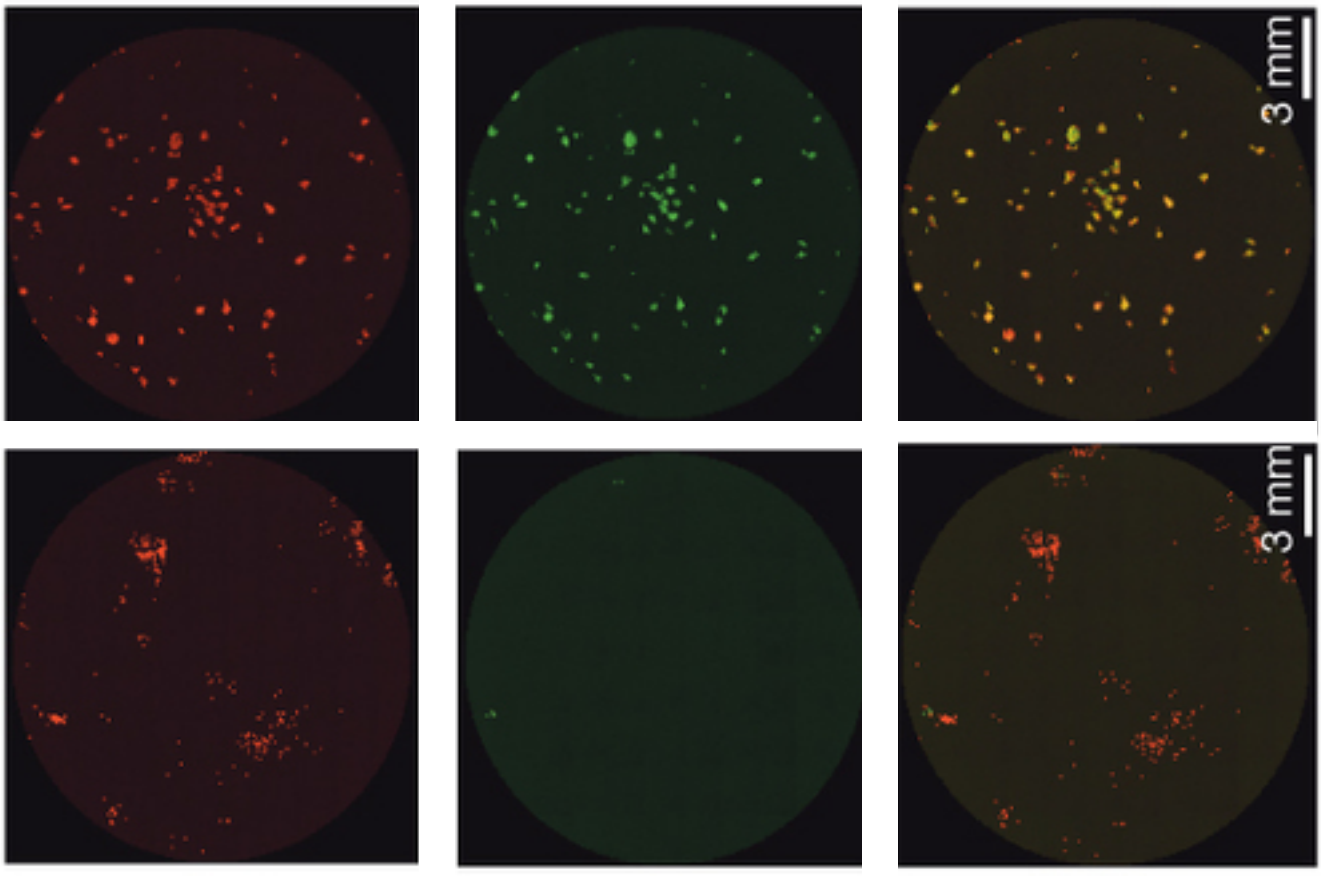
STEM CELL REPROGRAMMING MADE EASIER

Sep. 18, 2013

Embryonic stem cells have the enormous potential to treat and cure many medical problems. That is why the discovery that induced embryonic-like stem cells can be created from skin cells (iPS cells) was rewarded with a Nobel Prize in 2012. But the process has remained frustratingly slow and inefficient, and the resulting stem cells are not yet ready for medical use. Research in the lab of the Weizmann Institute's Dr. Yaqub Hanna, which appears today in Nature, dramatically changes that: He and his group revealed the "brake" that holds back the production of stem cells, and found that releasing this brake can both synchronize the process and increase its efficiency from around 1% or less today to 100%. These findings may help facilitate the production of stem cells for medical use, as well as advancing our understanding of the mysterious process by which adult cells can revert back into their original, embryonic state.



Left column: *This is the previous method for creating induced pluripotent stem cells (iPSCs).* ***Right column:*** *These are iPSCs produced with the new method developed by Dr. Hanna.* ***Top:*** *Skin cells (red); center: iPSCs from skin cells (green); bottom: superimposed top and center images. Skin cells that have been reprogrammed into iPSCs appear light yellow. Only a small percentage of the cells on the left have been reprogrammed, in contrast with the high success rate seen with the new method on the right. (Credit: Weizmann Institute of Science)*

Embryonic stem cells are those that have not undergone any "specialization"; thus they can give rise to any type of cell in the body. This is what makes them so valuable: They can be used, among other things, to repair damaged tissue, treat autoimmune disease and even grow transplant organs. Using stem cells

taken from embryos is problematic because of availability and ethical concerns, but the hopes for their use were renewed in 2006, when a team led by Shinya Yamanaka of Kyoto University discovered that it is possible to "reprogram" adult cells. The resulting cells, called "induced pluripotent stem cells" (iPSCs), are created by inserting four genes into their DNA. Despite this breakthrough, the reprograming process is fraught with difficulty: It can take up to four weeks; the timing is not coordinated among the cells; and less than one percent of the treated cells actually end up becoming stem cells.

Hanna and his team asked: What is the main obstacle -- or obstacles -- that prevent successful reprograming in the majority of cells? In his postdoctoral research, Hanna had employed mathematical models to show that a single obstacle was responsible. Of course in biology, Hanna is the first to admit, experimental proof is required to back up the models. The present study not only provides the proof, it reveals the identity of that single obstacle and shows that removing it can dramatically improve reprograming.

Hanna's group, led by Dr. Noa Novershtern, Yoach Rais, Asaf Zviran and Shay Geula of the Molecular Genetics Department, together with members of the genomics unit of the Institute's Israel Structural Proteomics Center, looked at a certain protein, called MBD3, whose function was unknown. MBD3 had caught their attention because it is expressed in every cell in the body, at every stage of development. This is quite rare: In general, most types of proteins are produced in specific cells, at specific times, for specific functions. The team found that there is one exception to the rule of universal expression of this protein: the first three days after conception. These are exactly the three days in which the fertilized egg begins dividing, and the nascent embryo is a growing ball of pluripotent stem cells that will eventually supply all the cell types in the body. Starting on the fourth day, differentiation begins and the cells already start to lose their pluripotent status. And that is just when the MBD3 proteins first appear.

This finding has significant implications for the producing iPSCs for medical use. Yamanaka used viruses to insert the four genes but, for safety reasons, these are not used in reprograming cells to be used in patients. This gives the process an even lower success rate of only around a tenth of a percent. The researchers showed that removing MBD3 from the adult cells can improve efficiency and speed the process by several orders of magnitude. The time needed to produce the stem cells was shortened from four weeks to eight days. As an added bonus, since the cells all underwent the reprograming at the same rate, the scientists will now be able, for the first time, to actually follow it step by step and reveal its mechanisms of operation. Hanna points out that his team's achievement was based on research into the natural pathways of embryonic development: "Scientists investigating reprograming can benefit from a deeper understanding of how embryonic stem cells are produced in nature. After all, nature still makes them best, in the most efficient manner."

Journal Reference:
Yoach Rais, Asaf Zviran, Shay Geula, Ohad Gafni, Elad Chomsky, Sergey Viukov, Abed AlFatah Mansour, Inbal Caspi, Vladislav Krupalnik, Mirie Zerbib, Itay Maza, Nofar Mor, Dror Baran, Leehee Weinberger, Diego A. Jaitin, David Lara-Astiaso, Ronnie Blecher-Gonen, Zohar Shipony, Zohar Mukamel, Tzachi Hagai, Shlomit Gilad, Daniela Amann-Zalcenstein, Amos Tanay, Ido Amit, Noa Novershtern, Jacob H. Hanna. *Deterministic direct reprogramming of somatic cells to pluripotency. Nature, 2013; DOI: 10.1038/nature12587*

EMBRYONIC STEM CELLS PRODUCED IN LIVING ADULT ORGANISMS

Centro Nacional de Investigaciones Oncologicas (CNIO)

Sep 11, 2013

A team from the Spanish National Cancer Research Centre (CNIO) has become the first to make adult cells from a living organism retreat in their evolutionary development to recover the characteristics of embryonic stem cells.

Researchers have also discovered that these embryonic stem cells, obtained directly from the inside of the organism, have a broader capacity for differentiation than those obtained via in vitro culture. Specifically, they have the characteristics of totipotent cells: a primitive state never before obtained in a laboratory.
The study, carried out by CNIO, was led by Manuel Serrano, the director of the Molecular Oncology Programme and head of the Tumoural Suppression Laboratory. The study was supported by Manuel Manzanares's team from the Spanish National Cardiovascular Research Centre (CNIC).

Embryonic stem cells are the main focus for the future of regenerative medicine. They are the only ones capable of generating any cell type from the hundreds of cell types that make up an adult organism, so they are the first step towards curing illnesses such as Alzheimer, Parkinson's disease or diabetes. Nevertheless, this type of cell has a very short lifespan, limited to the first days of embryonic development, and they do not exist in any part of an adult organism. One of the greatest achievements in recent biomedical research was in 2006 when Shinya Yamanaka managed to create embryonic stem cells (pluripotent stem cells, induced in vitro, or in vitro iPSCs) in a laboratory from adult cells, via a cocktail of just four genes. Yamanaka's discovery, for which he was awarded the Nobel Prize in Medicine in 2012, opened a new horizon in regenerative medicine.

CNIO researchers have taken another step forward, by achieving the same as Yamanaka, but this time within the same organism, in mice, without the need to pass through in vitro culture dishes. Generating these cells within an organism brings this technology even closer to regenerative medicine.

The first challenge for CNIO researchers was to reproduce the Yamanaka experiment in a living being. They chose a mouse as a model organism.

Using genetic manipulation techniques, researchers created mice in which Yamanaka's four genes could be activated at will. When these genes were activated, they observed that the adult cells were able to retreat in their evolutionary development to become embryonic stem cells in multiple tissues and organs.

María Abad, the lead author of the article and a researcher in Serrano's group, said: "This change of direction in development has never been observed in nature. We have demonstrated that we can also obtain embryonic stem cells in adult organisms and not only in the laboratory".

Manuel Serrano added that: "We can now start to think about methods for inducing regeneration locally and in a transitory manner for a particular damaged tissue". Stem cells obtained in mice also show totipotent characteristics never generated in a laboratory, equivalent to those present in human embryos at the 72-hour stage of development, when they are composed of just 16 cells.

In comparison with the cells obtained with the technique developed by Yamanaka, the stem cells obtained by CNIO therefore represent an even earlier embryonic state, with greater capacity for differentiation. The authors were even able to induce the formation of pseudo-embryonic structures in the thoracic and abdominal cavities of the mice. These pseudo-embryos displayed the three layers typical of embryos (ectoderm, mesoderm and endoderm), and extra-embryonic structures such as the Vitelline membrane and even signs of blood cell formation.

"This data tell us that our stem cells are much more versatile than Yamanaka's in vitro iPSCs, whose potency generates the different layers of the embryo but never tissues that sustain the development of a new embryo, like the placenta", said the CNIO researcher. The authors emphasise that the possible therapeutic applications of their work are still distant, but they admit that, without doubt, it might mean a change of direction for stem cell research, for regenerative medicine or for tissue engineering.

"Our stem cells also survive outside of mice, in a culture, so we can also manipulate them in a laboratory", said Abad, adding that: "The next step is studying if these new stem cells are capable of efficiently generating different tissues such as that of the pancreas, liver or kidney".

Watch Video in Original Article Below:
http://phys.org/news/2013-09-embryonic-stem-cells-adult.html
Paper: dx.doi.org/10.1038/nature12586