Safer stem cells for therapy

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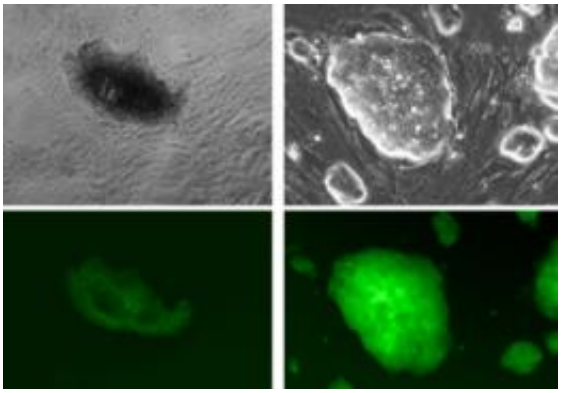

An iPS colony under bright light (top) and under UV light, showing activation of a green fluorescent reporter that specifically lights up in pluripotent cells. Courtesy Konrad Hochedlinger

(PhysOrg.com) -- When stem cell researchers in Japan and the United States announced in 2007 that they had developed long-sought methods to return fully developed adult human cells to an embryonic-like state, the world of stem cell research was turned upside down.

Media reports and conservative politicians prematurely hailed the discovery as a way to end the debate over the use of human embryonic stem cells. The discovery seemed to promise a way to produce endless supplies of stem cells that could be used to understand and treat a host of degenerative diseases including Alzheimer's, Parkinson's, diabetes, heart disease, and ALS, or Lou Gehrig's disease.

Predictably, the scientific reality has proven to be far more complicated than the wishes of patients, politicians, and researchers.

In order to produce what are called induced pluripotent stem cells - iPS - researchers had to use combinations of genes, some of which can induce the development of cancer. The question is how those genes can be eliminated and still produce cells that can ultimately become one of the 220 cell types in the body, the building blocks for all our organs.

“It’s complicated,” says Konrad Hochedlinger, a Harvard Stem Cell Institute (HSCI) principal faculty member and an assistant professor in Harvard’s new Department of Stem Cell and Regenerative Biology (SCRB). And as Amy Wagers, who has the same HSCI and SCRB titles, adds, “Stem cell biology is a very young field. There are new discoveries happening all the time.”

Recently, in fact, researchers at HSCI and Harvard-affiliated McLean Hospital created induced pluripotent stem cells (iPS) without inserting foreign genes, an advance that may ultimately accelerate therapeutic development by making the resulting cells safer for use in humans.

The team, led by Kwang-Soo Kim of HSCI, McLean, and Harvard Medical School, created its iPS cell line by bathing adult skin cells — fibroblasts, the cells that produce collagen and aid in wound healing — in proteins that previous experiments had shown would reprogram them to the embryonic stem cell-like state of pluripotency.

“The way iPS cells were made initially was quite challenging,” says Hochedlinger, a leader in iPS creation. It involved inserting four genes known to be active in embryonic stem cells into differentiated (adult) skin cells, such as fibroblasts, in mice. “Retroviruses were the shuttle system for the genes,” he says. Retroviruses are viruses that, by definition, penetrate the cell, inserting their DNA permanently in the

process. “And you don’t want to have viruses inside your cells,” Hochedlinger says, because tissues created from the iPS cells would also contain these extra viral genes, some of which are implicated in cancer, rendering them unsafe for use in tissue replacement therapy.

In the beginning...

Researchers first addressed the problem by finding a method that would allow them to insert the genes only temporarily; they used adenoviruses, which are associated with usually minor respiratory illnesses such as the common cold. “Adenoviruses enter the cell, deliver the genes, but never insert themselves into the cell’s DNA material,” Hochedlinger explains.

For at least three decades, scientists have been working to understand and control both the process of generating stem cells and the ways in which the most basic form of stem cells - called pluripotent - are able to differentiate into any form of body tissue, from blood, to heart, to nerve cell. Normally, pluripotent stem cells only exist in a very early stage of an embryo called the blastocyst.

The blastocyst is formed when egg and sperm combine to form a single cell with the potential to form an entire organism - a “totipotent” cell. After a few days, and several cycles of cell division, the single cell is transformed into the half-hollow sphere of cells that is the blastocyst.

Inside the body, where environmental factors trigger differentiation, the blastocyst’s pluripotent inner population of cells exists only transiently. “So it’s a very brief time window during development when we have pluripotent stem cells,” Hochedlinger says. “There’s no way we can derive embryonic stem cells from ourselves, unless we manipulate the system artificially.”

“If you place this blastocyst in culture [a petri dish in a lab], its inner

mass cells would not do much," says Hochedlinger. "They'll probably just poop out. They'll die." To keep them alive and proliferating, researchers must add a cocktail of growth factors important for cell division. The added growth factors arrest the cells in their pluripotent stages.

"Like a tumor, these cells grow and grow and, within four or five days, become what we call a permanent pluripotent embryonic cell line," an immortal line of stem cells that do not develop further, and have no instructions except dividing. "But you have to push these undifferentiated cells into becoming the particular body cells you want to produce, say nerve cells or liver cells, by exchanging growth factors important for division with factors that are important for the development of the cell type you want."

Another way to obtain pluripotent embryonic stem cells from a blastocyst is by nuclear transfer, popularly known as cloning. "It works in mice," Hochedlinger explains. "Theoretically one could take skin cells, extract their nucleus [which contains DNA], put it into egg cells that have been devoid of their own nucleus, and make pluripotent embryonic stem cell lines out of that. This hasn't worked in humans yet," he adds.

An alternative to generating pluripotent embryonic stem cells is coaxing already adult cells into becoming pluripotent again. "We take our skin cells, then introduce genes into them with reprogramming factors that convert these cells into pluripotent entities that are very similar to pluripotent embryonic cells - without ever having gone through an actual embryonic stage," Hochedlinger says. This is what researchers call "reprogramming," their equivalent achievement of the medieval alchemists' never-realized dream of turning lead into gold.

Nuclear transfer and the creation of patient-specific stem cell lines

could, Hochedlinger says, be used to treat diseases that are not caused by genetic mutations. “Let’s say, for example, that you drink a lot of alcohol, and you destroy your liver. You don’t necessarily have a genetic mutation; you did this by yourself. Your DNA is still OK,” he says. “So we can get skin cells or any other adult cells easily accessible from your body, blood cells, say, reprogram them into iPS cells,” culture them in large quantities in the lab, and “then we could transplant those cells back into your body,” Hochedlinger explains, adding that the idea is to transfer normal cells back into patients whose cells are defective.

Induced pluripotency is a technology that some stem cell researchers regard as something short of revolutionary within the field of stem cells. It’s an idea that some researchers believe started with a creature named Dolly.

Hello, Dolly

As Hochedlinger explains, induced pluripotent stem cells were the product of two main discoveries. The first one was the generation of Dolly the sheep, a light-brown-haired celebrity clone who captured the public’s imagination. Dolly died of progressive lung disease at merely six years old, in 2003.

“Dolly’s birth,” Hochedlinger says, “demonstrated that you can take an adult mammary gland cell and actually turn back time on it into a pluripotent embryonic state to generate an entire animal from it.” In other words, cloning showed that cells could be “reprogrammed”: An adult cell could be turned into a pluripotent embryonic cell again.

Prior to the announcement of Dolly’s birth, even many biologists thought mammalian cell reprogramming was impossible, says Hochedlinger. The discovery, a year later, of human pluripotent stem cells taken directly from embryos taught researchers that it was also possible to maintain a

pluripotent embryonic cell line culture from human cells.

“The possibility to reprogram led to the possibility to take a patient-specific cell, like a patient’s skin cell, and reverse it into a pluripotent embryonic stem cell that can be used for therapeutic purposes,” Hochedlinger says.

What many in the field refer to as the real breakthrough, however, took place merely two years ago, in 2007, when a team led by Japanese orthopedic surgeon-turned-stem-cell-researcher Shinya Yamanaka, working at Japan's Kyoto University, created the first iPS cells from human tissue. Yamanaka’s finding was hailed by those who oppose embryonic stem cell research for religious reasons as a way to eliminate the need to destroy embryos.

The great pretender?

There are major questions still, says researcher Ole Isacson, professor of neurology at the Division of Medical Science at Harvard. “Taking an example from the context of neuroscience and neurology, the first question is whether a neuron generated from an iPS cell line is really equivalent to a neuron differentiated within the body. Moreover, is the iPS cell actually equivalent to the pluripotent stem cell derived from an embryo?” asks Isacson.

So far, argues Hochedlinger, all the evidence indicates that these iPS cells are, at least, very similar to pluripotent embryonic stem cells that come from a blastocyst (embryo). “They grow indefinitely in culture, and they can be coaxed in vitro, when you expose them to growth factors, into becoming other cell types. But we don't yet know if iPS cells and pluripotent embryonic cells are truly identical.”

The process can be inefficient, as well. Says Hochedlinger, “The cells

that we end up with are often not equivalent to a cell that normally develops in the body, for whatever reason; probably because they don't go through all the normal stages of development that they normally go through in the body."

And there are other problems. "These cells have the potential to make any tissue in the body, but at the moment, we still have to figure out how to instruct them as to make any particular cell that you want, without them making cells that you don't want," says Wagers.

"Also, because they divide indefinitely in culture," says Hochedlinger, "in experiments where you transplant them back into mice, they very often develop into tumors because it's difficult to get rid of residual undifferentiated cells in your culture." Only one cell that remains undifferentiated among the rest, and keeps dividing, could grow into a teratoma, a mass of cells that never got reprogrammed.

The recent report from HSCI researchers at McLean Hospital that they had produced iPS cells using proteins rather than genes offers promise as a safer method. Instead of inserting genes into the cell's DNA, inducing the cell to make the proteins that will reprogram it, Kim and his colleagues attached a molecule that specializes in penetrating cell membranes to insert the needed reprogramming proteins. Once this molecule, called a "cell-penetrating peptide," was attached, the proteins were able to enter the cell and begin reprogramming it.

For Kim, however, significant hurdles do remain. While foregoing the need to insert genes may eliminate many important concerns, the effects of the proteins themselves have to be thoroughly understood, since proteins fulfill many roles in the body and are not inherently "safe."

Kim has been working on stem cells for about a decade and has focused his efforts to reprogram stem cells using proteins because he feels that

cells created by gene insertion would not be useful in therapy.

“You don’t want these cells in your body,” Kim says. “I strongly believe this is a safer way and is medically and clinically feasible.”

The process used by his team and colleagues still needs improvement, Kim concedes, as it is much less efficient than the gene-insertion process, producing about 10 times fewer cells. He pinpointed one part of the process that can be improved immediately, saying his team used an extract of pluripotent embryonic stem cells as a source for proteins, rather than purified solutions of the proteins themselves. Using purified proteins, he says, will likely improve efficiency.

As Kim explains, the first hint that the research team had created iPS cells came around Christmas 2008. He and colleagues have spent the past several months checking their results and conducting tests to determine the character of the cells. The cells they created, he said, have passed every known test that indicates they are iPS cells.

A future reprogrammed

Hochedlinger believes he and his colleagues will be modeling diseases in a petri dish within the next five or 10 years. “We will be able to recapitulate the course of a disease and find tracks that can possibly fix the problems,” he says. “Modeling a disease will give us the opportunity for drug discovery,” adds Wagers.

“We could take skin cells from patients with ALS, for example,” says Isacson, “make them into iPS cells, reprogram them into becoming neurons over a few weeks in culture, and then see if these cells show some sign of the disease. If they do, drug companies can take the cells and start testing new drugs.”

“It may not be for all diseases,” Hochedlinger says, “but even just one out of 10 will be very good.”

Neither Hochedlinger nor Wagers believes this will happen soon. “That’s more like 20 years down the road, mostly for safety reasons,” Hochedlinger says.

Finally, will either pluripotent embryonic stem cells or iPS cells emerge as the 'tools' of choice for stem cell researchers? Hochedlinger doesn’t think so. “It's unclear at this point who's the winner; we still need to work on both types of stem cells.”

“Both pluripotent embryonic stem cells and iPS cells are equally tumorigenic, and rare undifferentiated cells could give rise to a teratoma from both sources when transplanted into a patient,” he adds. “The main advantage of iPS cells is that they can be derived from any living individual, especially patients. Pluripotent embryonic stem cells, on the other hand, are much better characterized than iPS cells and still represent the gold standard for a pluripotent cell line.”

Provided by Harvard University (news : web)