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Researchers Devise New Technique for Creating Human Stem Cells

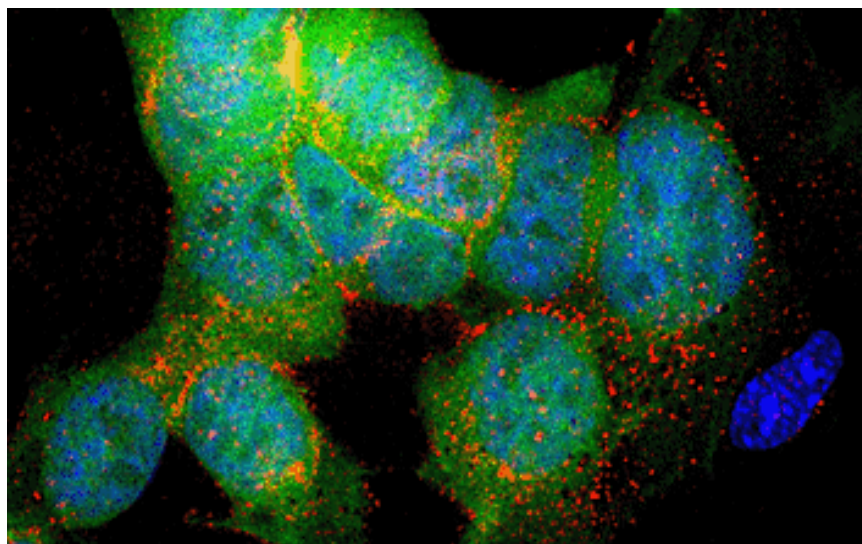


Image Title: The image shows human embryonic stem cells stained for a characteristic marker protein (Tra1-60, red dots). When adult skin cells are fused with embryonic stem cells, the hybrid cells (not shown) re-express this marker, suggesting that the cells have reverted to the embryonic state. - Chad A. Cowan, HHMI at Harvard University, for Science.

Researchers have developed a new technique for creating human embryonic stem cells by fusing adult somatic cells with embryonic stem cells. The fusion causes the adult cells to undergo genetic reprogramming, which results in cells that have the developmental characteristics of human embryonic stem cells. The new technique may permit scientists to derive new human embryonic stem cell lines without the need to use human embryos.

This approach could become an alternative to somatic cell nuclear transfer (SCNT), a method that is currently used to produce human stem cells. SCNT involves transferring the nuclei of adult cells, called somatic cells, into oocytes in which scientists have removed the nuclei.

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- Douglas A. Melton

The researchers said that — while the technique might one day be used along with SCNT, which involves the use of unfertilized human eggs — technical hurdles must be cleared before the new technique sees widespread use. It is more likely that the new technique will see immediate use in helping to accelerate understanding of how embryonic cells “reprogram” somatic cells to an embryonic state.

The researchers published their findings in the August 26, 2005, issue of the journal *Science*. Senior author Kevin Eggan and Howard Hughes Medical Institute investigator Douglas A. Melton, both at Harvard University, led the research team, which also included Harvard colleagues Chad Cowan and Jocelyn Atienza.

In theory, researchers can induce embryonic stem cells to mature into a variety of specialized cells. For that reason, many researchers believe stem cells offer promise for creating populations of specialized cells that can be used to rejuvenate organs, such as the pancreas or heart, that are damaged by disease or trauma. Stem cells also provide a model system in which researchers can study the causes of genetic disease and the basis of embryonic development.

Eggan, Melton and their colleagues decided to pursue their alternative route after other researchers had shown that genetic reprogramming can occur when mouse somatic cells are fused to mouse embryonic stem cells. The scientists knew that if their studies were successful, it would provide the research community with a new option for producing reprogrammed cells using embryonic stem cells, which are more plentiful and easier to obtain than unfertilized human eggs.

In the studies published in *Science*, the researchers combined human fibroblast cells with human embryonic stem cells in the presence of a detergent-like substance that caused the two cell types to fuse. The

researchers demonstrated that they had achieved fusion of the two cell types by searching the fused cells for two distinctive genetic markers present in the somatic fibroblast and stem cells. The researchers were also able to further confirm that fusion occurred by studying the chromosomal makeup of the fused cells. Their analyses showed that the hybrid cells were “tetraploid” - meaning they contained the combined chromosomes of both the somatic cells and the embryonic stem cells.

One of the key findings from the study was that the fusion cells have the characteristics of human embryonic stem cells. “Our assays showed that the hybrid cells, unlike adult cells, showed the development potential of embryonic stem cells,” said Eggen.

“We found they could be induced to mature into nerve cells, hair follicles, muscle cells and gut endoderm cells. And, since these cell types are derived from three different parts of the embryo, this really demonstrated the ability of these cells to give rise to a variety of different cell types.”

Furthermore, Eggen noted that genetic analyses of the fused cells revealed that the somatic cell genes characteristic of adult cells had all been switched off, while those characteristic of embryonic cells had been switched on. “With the exception of a few genes one way or the other — which is perhaps because these cells are now tetraploid — the hybrid cells are indistinguishable from human embryonic stem cells,” he said.

“The long term goal for this experiment was to do cell fusion in a way that would allow the elimination of the embryonic stem cell nucleus to create an embryonic stem cell from the somatic cell,” said Melton. “This paper reports only the first step toward that goal, because we end up with a tetraploid cell. So, while this does not obviate the need for human oocytes, it demonstrates that this general approach of cell fusion is an interesting one that should be further explored.”

The researchers also performed fusion experiments using pelvic bone cells as the somatic cells and a different human embryonic cell line, to demonstrate that their technique was not restricted to one adult cell type or embryonic cell line.

In both cases, the researchers observed extensive reprogramming of the somatic cells. “We were surprised at how complete the reprogramming was,” said Eggen. “I think we were expecting that there would be more 'memory' of the adult state than the embryonic in the hybrid cells. It was quite clear that

when we looked at these hybrid cells, they had completely reverted to an embryonic state.”

Melton said that the remaining technical hurdle is figuring out a way to eliminate the embryonic stem cell nucleus in the hybrid cell, causing it to have a normal number of chromosomes. One problem, said Melton, is that the nucleus in stem cells is large, occupying nearly the entire cell. Thus, it is not practical to physically extract the nucleus, as is currently done with oocytes, which have a relatively small nucleus. An alternative approach of destroying the embryonic stem cell nucleus with chemicals or radiation would induce the cell's suicide program, called apoptosis, he said.

Melton emphasized that “at this stage in our understanding, the hard fact is that the only way to create an embryonic stem cell from a somatic cell is by nuclear transfer into oocytes. Taking advantage of this current capability — such as colleagues in South Korea and other countries are doing — is critical if we are to maintain the progress necessary to realize the extraordinary clinical potential of this technology.”

Eggan added that the most realistic current promise of the fusion technique is in studying the machinery of genetic reprogramming of somatic cells by embryonic cells. “It is extremely difficult to study the reprogramming process using eggs, because in the case of humans it is very difficult to obtain eggs in any quantity and difficult or impossible to genetically manipulate them,” he said. “But embryonic stem cells can be grown in large quantities. We can isolate the components of the reprogramming machinery, and we can genetically manipulate the cells to analyze the reprogramming process.”