Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2]CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024

Abstract

Differentiated cells can be reprogrammed to an embryonic-like state by transfer of nuclear contents into oocytes or by fusion with embryonic stem (ES) cells. Little is known about factors that induce this reprogramming. Here, we demonstrate induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, Oct3/4, Sox2, c-Myc, and Klf4, under ES cell culture conditions. Unexpectedly, Nanog was dispensable. These cells, which we designated iPS (induced pluripotent stem) cells, exhibit the morphology and growth properties of ES cells and express ES cell marker genes. Subcutaneous transplantation of iPS cells into nude mice resulted in tumors containing a variety of tissues from all three germ layers. Following injection into blastocysts, iPS cells contributed to mouse embryonic development. These data demonstrate that pluripotent stem cells can be directly generated from fibroblast cultures by the addition of only a few defined factors.

Embryonic stem cells are cells that are isolated from approximately 5 day old embryos, when the embryo is a ball of cells. The cells of the young embryo have not developed into body stuctures yet, and have the ability to turn into any cell in the human body. This ability to turn into any cell is called **pluripotency**. Pluripotent stem cells can be isolated and coaxed in a laboratory to become any cell in the body. Many scientist and doctors are interested in human embryonic stem cells because they believe stem cells have the potential to treat many human diseases such as diabetes, spinal cord injury, and Parkinson's disease by coaxing stem cells to develop into the cells that are damaged in these conditions and transplanting them into patients. However, many people believe destroying a human embryo to create stem cells is immoral.

This paper by Shinya Yamanaka and colleagues describes the first time **somatic**, or body, cells were reprogrammed into embryonic stem cell-like cells and the methods that the authors used to discover how to do this. These embryonic stem cell-like cells are called **Induced Pluripotent**

Stem (iPS) Cells. Since this paper was published this method has been used to create iPS cells from many other species, most importantly humans. These cells behave very similarly to embryonic stem cells. If these cells are similar enough to embryonic stem cells they could potentially be used in stem cell research and therapies without having to destroy an embryo.

The researchers began by identifying 24 genes that are present in embryonic stem cells. The identity of a cell is determined by the types of genes that are expressed in the cell. The genes that are expressed in a stem cell cause it to be pluripotent. Yamanaka and his colleagues hoped that some of the 24 genes that they identified were what can be thought of as master regulators. A master regulator is a gene whose main function is to turn on many other genes. In other words, Yamanaka was looking for the gene or genes that turn on the many other genes that cause pluripotency.

To sort through these 24 genes Yamanaka developed a clever system. They had previously found a gene called Fbx15 that is expressed only in embryonic stem cells, but is not important for the function of the cell. They used biotechnology to replace the DNA sequence for the Fbx15 protein with two genes; one which causes a blue color and another gene that makes a cell resistant to a drug. Cells that would normally express the pluripotency gene Fbx15 would now live if exposed to the drug.

Using this system they tested each of the 24 genes. They engineered **retroviruses** (viruses that insert their genetic code into the cell's DNA) to express one of the 24 genes and **transfected** cultured mouse **fibroblasts** (found in the skin) with each virus. Unfortunately not a single gene caused Fbx15 to be activated, and no cells became blue or drug-resistant. None of the 24 genes was able to cause pluripotency alone. The researchers then thought it was maybe a combination of multiple genes that caused pluripotency. To test this they transfected cells with all 24 genes at once. This time they found several cells that were blue and drug resistant, looked and acted like stem cells, and expressed many other pluripotency genes.

So it appeared as though the researchers could make stem cell-like cells, but they did not know how many genes were needed to do this. They only knew some number between 2 and 24 were necessary, and they didn't know which ones. Testing every single combination would have been virtually impossible. Instead they tested what happened if they left each of the 24 genes out. They transfected fibroblast with 23 out of 24 genes. If one of the genes was necessary for making pluripotent cells, then no stem cell like cell would form without it. Using this method they identified 4 genes that were essential for inducing pluripotency, Oct3/4, Klf4, Sox2, and c-Myc. All of these genes are **Transcription Factors**, which are proteins which bind to specific regions of DNA and either suppress or activate the expression of other genes. These four genes are now commonly referred to as the Yamanaka Factors.

Using these four genes the researchers formed four cell lines that appeared to be similar to embryonic stem cells. They called these cells as iPS cells. They next performed tests to determine if the cells were actually embryonic stem cells. They used some tests normally applied to embryonic stem cells.

1. They analyzed the gene expression of the cell lines and compared them to both embryonic stem cells and fibroblasts (the cells that the iPS cells were formed from). IPS cells were not identical to either embryonic stem cells or fibroblasts. However, the iPS cells were much more similar to embryonic stem cells than to fibroblasts. Interestingly, they found that not all of the iPS cell lines were identical. Some appeared more embryonic stem cell-like than others.
2. An important test of embryonic stem cells can form tumors called **teratomas** when injected into a mouse. Teratomas contain all three **germ layers**. If a cell line can form all three cell layers it shows that the cells can develop into all cell types. They tested several iPS lines. Three of the four lines tested were able to form teratomas, indicating they were in fact pluripotent. One iPS line failed to differentiate and was therefore not pluripotent.
3. They also grew the cells in culture and found that three of the four cell lines **differentiated** (grew into mature cell types), another key characteristic of embryonic stem cells. The line that failed to form teratomas also did not differentiate in culture.
4. They also tested whether the iPS cells could form **chimeras**. Chimeras are animals that have cells of two or more genetic origins. The iPS cells were injected into mouse **blastocysts**. Then the blastocysts were transplanted into a female mouse and allowed to develop into a pregnancy. The iPS cells contained another colored gene called Green Fluorescent proteins, while the blastocystes did not have these genes. The resulting embryos were examined later and found to contain both green cells and cells lacking green. Both the iPS and the cells from the blastocyst had contributed to the structure of the embryo. This meant that the iPS cells were pluripotent and could grow normally in a developing animal. However, no animals were found that survived until birth. Chimeras formed from ES cells typically survive into adulthood. Later studies performed by this and other groups with iPS cells were able to create surviving adult animals.

In conclusion, the researchers were able to create embryonic stem cell-like cells from fibroblasts by expressing four regulatory genes; Oct3/4, Sox2, Klf4, and c-Myc. However, these iPS cells were not exactly identical to embryonic stem cells. Also, not all the iPS cell lines behaved the same. This is only the first paper describing this technique, and much more research is required to further understand the relationship between these cells and embryonic stem cells. A major concern of the authors is that the efficiency of making stem cells is exceedingly low. Only about 0.02% of cells transfected with the factors became iPS cells. They theorize that the cells that become iPS cells may be adult stem cells within the skin. Because an adult stem cell is

multipotent it is in theory is more similar to an embryonic stem cell than a somatic cell. It might be more easily transformed into an iPS cell than a somatic cell.