

## Human iPS Cells: a New Beginning for Modern Regenerative Medicine

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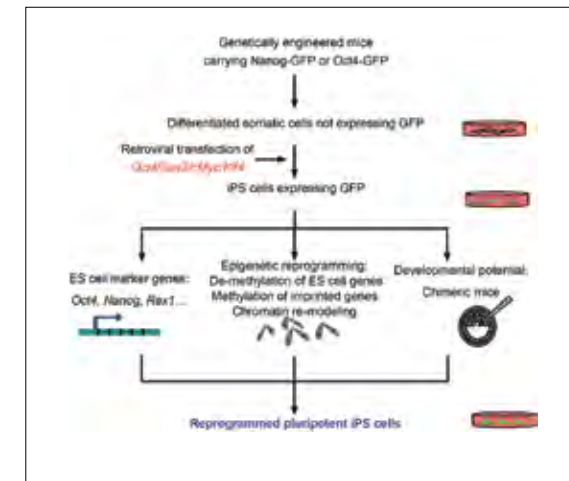


**About Author:** Dr. Junying Yu received BS degree in Plant Molecular and Developmental Biology from College of Life Science, Peking University in 1997 and graduated magna cum laude in the Advanced Science Program in 1996. She received Ph.D. degree in Cell and Molecular Biology in 2002 from University of Pennsylvania. From 2003-2005, she conducted post-doctoral training in Wisconsin National Primate Research Center, University of Wisconsin under supervision of Professor James Thomson. Dr Yu is currently an Assistant Scientist in Wisconsin National Primate Research Center. Dr Yu has published a dozen of articles and is a co-inventor of two stem cell related patents. Her recent article on somatic cell reprogramming published in *Science* has been listed as the #2 scientific breakthrough among *Science Magazine's* "Breakthrough of the Year" issue in 2008 and listed as the #1 on *TIME Magazine's* Top 10 Scientific Discoveries in 2007.

Since the dawn of human history, people have been fascinated with the idea of immortality. Stories of "the Fountain of Youth," a well or spring that has tremendous healing power, for example, have flourished over centuries. Reflected in modern science, the successful derivation of human induced pluripotent stem (iPS) cells directly from human somatic cells, perhaps represents our first attempt to make this dream a reality.

Mammalian development is executed as accurately coordinated temporal differentiation events. It starts as a one-cell embryo, which is the true totipotent cell, as on its own, it can give rise to all cells required for a new life. As the one-cell embryo divides, it forms morula, each cell of which is still capable of forming a new life. The first differentiation event occurs when the outside layer of cells in morula delaminate from the rest of the embryo to form trophoblast. At this stage, the embryo is referred to as a blastocyst. The cells inside the blastocyst (inner cell mass or ICM) are the ones that will give rise to all cells of the adult body (pluripotent), while the outside trophoblast, along with some ICM cells, will form placenta. As development proceeds, cells become more and more restricted in their ability to differentiate into additional cell types. In adults, stem cells are found in several tissues/organs such as hematopoietic stem cells in bone marrow and neural stem cells in brain. These adult stem cells are responsible for normal homeostasis of their corresponding tissues/organs. However, they are present at a very limited number in our bodies, and normally only differentiate into cells specific to the tissue/organ where they reside, not others. These limitations are some of the major causes for our bodies' extremely poor ability to repair damaged tissues and recover cell loss occurring in various diseases or during the aging process. One solution for the cure of many prevalent illnesses such as Parkinson's, diabetes, and heart failure is to replace diseased/dead cells with healthy cells through transplantation therapies. But there is a severe shortage of source material for tissues used in transplantation. The establishment of human embryonic stem (ES) cells in 1998<sup>(1)</sup> seemed to be able to offer a great solution to this problem as these cells are capable of indefinite proliferation, and have the ability to differentiate into all cell types of the body – an essentially unlimited cell supply for transplantation.

Human ES cells are derived from the ICM cells of blastocysts. The derivation process usually involves the destruction of embryos, generating both ethical and moral concerns over their use. Moreover, there is one major hurdle to their use in transplantation: immune rejection. This led to many efforts in deriving patient-specific human ES cell lines. One method is through somatic cell nuclear transfer (SCNT). The live-birth of Dolly in 1997 changed the mindset of biologists<sup>(2)</sup>. It proved that mammalian development is not unidirectional, as previously thought. The identity of adult somatic cells can be erased and reverted to an earlier developmental state (totipotent or pluripotent). The application of this technology in humans, however, remains very problematic despite the recent success in primates<sup>(3)</sup>. Its low efficiency and extremely limited supply of human eggs would make this technology unaffordable by most people. Moreover, it still involves the creation



and destruction of embryos, which is both ethically and morally challenging. We have taken an alternative approach to address this problem. Mouse ES cells, similar to mouse eggs, were able to revert the fully differentiated somatic cells to a state of pluripotency upon cell-cell fusion<sup>(4)</sup>. This suggests that ES cells also contain reprogramming factors sufficient to establish pluripotency in somatic cells. Since only pluripotent cells such as ES cells, not others, have such ability, genes specifically expressed in these cells are likely important for reprogramming. After confirming that human ES cells, similar to mouse ES cells, were also capable of reprogramming somatic cells<sup>(5)</sup>, we established a screening system to identify genes that could revert the fate of human somatic cells, and successfully found a combination of four genes (*OCT4*, *SOX2*, *NANOG* and *LIN28*) that could establish pluripotency in fibroblasts obtained from human fetal lung, newborn foreskin and adult abdomen. Among these four genes, *OCT4* and *SOX2* appeared to be essential, while *NANOG* and *LIN28* were only beneficial<sup>(6)</sup>. Independently, Yamanaka's group also successfully established iPS cells from human skin cells using a different gene combination (*OCT4*, *SOX2*, *c-Myc* and *KLf4*)<sup>(7)</sup>.

The human iPS cells share two key features with human ES cells: indefinite expansion potential and the ability to differentiate into all cell types of the body. Though detailed studies and comparison between human iPS and ES cells are yet to be carried out, these two key features make human iPS cells quite useful for many applications. The absence of embryo involvement during iPS cell derivation eliminates any ethical or moral concerns. And, in contrast to the SCNT technology, the simplicity of the derivation process would allow more researchers around the world to pursue iPS cell research, which could significantly facilitate the translation of research into clinics.

The success of human iPS cell derivation is only the beginning. There are many questions that need to be addressed before any application of iPS cells can be realized. One immediate concern is the transgene delivery

system. Currently, retroviral and lentiviral vectors are employed to deliver reprogramming genes into human somatic cells. These vectors are integrated into the somatic genome, which may cause harmful mutations. And the residual transgene expression, especially with lentiviral vectors, would interfere with the ability of human iPS cells to differentiate. Additionally, possible reactivation of transgene expression during differentiation of human iPS cells would lead to unwanted effects such as tumor formation. Alternative approaches are needed to derive iPS cells, such as the use of a transient expression system. Only when human iPS cells are derived with the viral vectors and transgenes removed will detailed studies of these cells be informative. And with such an improved protocol, human iPS cells can be derived from individuals susceptible to various diseases, which could provide extremely valuable tools to study disease occurrence and to develop novel therapies. Human iPS cells derived from a diversity of genetic backgrounds can also be used for drug screening in vitro. More importantly, it could prove to be extremely useful in establishing a large bank of pluripotent stem cells with HLA haplotypes representing the genetic diversity of a population for transplantation therapies, without destroying any embryos.

Besides these immediate perceivable applications, the implication of the successful human iPS cell derivation is by far the most exciting of all. The identities of adult human somatic cells normally are very stable, which is essential for them to carry out their functions in the body. These cell states, however, can now be erased and reverted to a "ground state," a state of pluripotency, simply by overexpressing a few transgenes. This suggests that it might be equally possible to change one type of cell directly to another type (transdifferentiation), or to revert fully differentiated cells back to precursors (dedifferentiation), capable of active cell division and further differentiation through simple genetic manipulation. Moreover, it might be possible to identify small molecules that could mimic the functions of reprogramming genes, thus achieving reprogramming without genetic modifications. The key to "the Fountain of Youth" may well lie in these small reprogramming compounds.

### References

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