**Title:**

YOUR INNER HEALERS. By: Hochedlinger, Konrad, Scientific American, 00368733, May2010, Vol. 302, Issue 5

**Database:**

MasterFILE Premier

**HTML Full Text**

**YOUR** **INNER** **HEALERS**

**Section:**

MEDICINE

**Reprogramming cells from your own body could give them the therapeutic power of embryonic stem cells, without the political controversy**

I remember my excitement one morning in the winter of 2006 when I peered through a microscope in my laboratory and saw a colony of **cells** that looked just like embryonic **stem** **cells**. They were clustered in a little heap, after dividing in a petri dish for almost three weeks. And they were glowing with the same colorful fluorescent markers scientists take as one sign of an embryonic **cell's** "pluripotency"--its ability to give rise to any type of tissue in an organism's body. But the **cells** I was looking at did not come from any embryo: they were regular adult mouse **cells** that had seemingly been rejuvenated by the addition of a simple cocktail of genes.

Could it really be so easy to roll back the internal clock of any mammalian **cell** and return it to an embryonic state? I was not the only one wondering at the time. Shinya Yamanaka of the University of Kyoto and his colleagues had just published a groundbreaking study in August 2006 that revealed their formula for creating what they called **induced** **pluripotent** **stem** **cells** (iPSCs) from the skin **cells** of mice. Researchers had been struggling for years to understand and control the enormous **potential** of embryonic **stem** **cells** to produce customized tissues for use in medicine and research--as well as contending with political and ethical controversies over the use of embryos, scientific setbacks and false hopes generated by previous "breakthroughs" that did not pan out. So **stem** **cell** scientists were surprised and a little bit skeptical of the Japanese group's results at first. But that morning in the lab, I could see firsthand the results of following Yamanaka's recipe.

Other scientists were also able to reproduce his achievement, and improved techniques for making and testing iPSCs have come rapidly over the past few years. Today thousands of scientists worldwide are working to develop the **potential** of iPSCs to help in understanding and treating human diseases that have so far defied cures, such as type 1 diabetes, Alzheimer's disease and Parkinson's disease. The possibility of changing a **cell's** identity just by delivering a few select genes has transformed the way scientists think about human development as well.

Throughout history people have dreamed of finding a Fountain of Youth to escape the consequences of aging and disease, and the ability to return an adult body **cell** to an embryonic state would certainly appear to be as close as humanity has come to that fantasy so far. Of course, the technology is still in its infancy. Many important questions must be answered before anyone can say whether iPSCs will change the practice of medicine or even whether they will actually prove equivalent to the more controversial embryonic **stem** **cells**.

**Primordial Power**

To understand the hopes inspired by the discovery of iPSCs, one must return to what makes embryos so special. Current iPSC studies rely heavily on techniques and concepts developed in work with embryonic **cells** over the past 30 years, particularly the phenomenon of pluripotency. Mammalian development is normally a one way-street, where **cells** become progressively more specialized and less versatile with time, a process called differentiation. Only during a brief window very early in development do all the **cells** within an embryo possess the ability to become any of the 220 **cell** types in the human body. Extracting those **cells** and growing them in culture gives rise to embryonic **stem** **cells**. The ability of true embryonic **stem** **cells** to indefinitely maintain their capacity to generate any tissue type defines the term "**pluripotent**."

Even in a late-stage embryo, **stem** **cells** have specialized to the extent that they can give rise only to specific families of **cell** types, such as those in muscle and bone. These **cells** are considered "multipotent," but they are no longer **pluripotent**. In an adult, all that remains of those precursors are so-called adult **stem** **cells** that replenish mature **cells** within a tissue. Blood **stem** **cells** continuously regenerate the 12 different blood and immune **cell** types, for example, and skin **stem** **cells** are responsible for re-growing our skin and hair every few weeks.

In mammals the one thing that never happens under normal circumstances is for a **cell** to dedifferentiate, that is, revert back to a more primitive type. Indeed, the only exception to this rule is cancer **cells**, which can become less differentiated than the tissue in which they first arise. Unfortunately, some cancer **cells** can also continue to divide endlessly, displaying an immortality similar to that of **pluripotent** **cells**.

Until recently, the only way to turn back the developmental clock of a normal adult **cell** was through elaborate manipulations to trick it **into** behaving like an embryonic **cell**, a process termed cellular reprogramming. The oldest approach to achieving reprogramming is somatic **cell** nuclear transfer, or "cloning," which involves injecting the genetic material from an adult **cell** **into** an egg **cell** whose own DNA has been removed. This DNA-egg hybrid then develops **into** an early-stage embryo from which **pluripotent** **stem** **cells** can be extracted.

Since the cloning of Dolly the sheep was revealed in 1997 and the first isolation of human embryonic **stem** **cells** in 1998, nuclear transfer has received considerable attention as a possible means of producing custom-tailored **pluripotent** **stem** **cells** to replace any tissue damaged through injury or disease. Poorly understood factors within the egg do seem to genuinely rejuvenate the genetic material of the adult donor **cell**--even telomeres, the caps protecting the ends of chromosomes that wear away with age, are restored to a youthful state. Yet despite progress with animals, attempts to produce human embryonic **stem** **cells** through cloning have remained unsuccessful.

Yamanaka and his group went around this impasse by taking a novel approach to turning adult **cells** directly **into** **pluripotent** **cells** without the use of eggs or embryos. Instead of introducing adult genetic material **into** an egg, they reasoned that introducing the genes normally active only in embryos **into** an adult **cell** might be sufficient to reprogram that **cell** **into** an embryolike state. Their first feat was to identify a cocktail of two dozen different genes that are turned on in **pluripotent** **cells** but silent in adult **cells**. When introduced **into** skin **cells** using retroviruses as delivery vehicles, these genes then almost magically reprogrammed the identity of the skin **cells** **into** that of **pluripotent** **cells**. With further experiments, Yamanaka then found that only four genes--Oct4, Sox2, Klf4 and c-Myc--were actually necessary to produce iPSCs.

As soon as several independent laboratories, including mine, successfully reproduced the results, this magic trick became a biological fact. By now about a dozen different adult **cell** types from a total of four different species (mouse, human, rat and monkey) have been reprogrammed **into** iPSCs, and certainly more will follow. The discovery of iPSCs is so thrilling to **stem** **cell** researchers because they can circumvent the technical complexities of cloning and avoid most of the ethical and legal constraints associated with human embryo research. This new **pluripotent** **cell** type is not without its own problems, however. Quality control and safety are the main focus of iPSC research right now, as scientists work to establish what these **cells** really are and what they are capable of doing.

**Identity Crisis**

Although iPSC colonies may **look** like embryonic **stem** **cells** under a microscope and may display the molecular markers associated with **pluripotent** **cells**, the unequivocal proof of their pluripotency comes from functional testing--can the **cells** do all the things a **pluripotent** **cell**, by definition, can do? Even embryo **cell** colonies can contain some dud **cells** that do not display the pluripotency of a true embryonic **stem** **cell**, and scientists have developed a few routine tests to gauge a **cell's** pluripotency. With increasing stringency, they are: the ability of **stem** **cells** to produce a wide variety of body **cell** types in a petri dish when exposed to the appropriate developmental cues; the ability of **stem** **cells** to produce a teratoma (a type of tumor containing **cells** from all embryonic tissue lineages) when injected under the skin of a mouse; and the capacity, when injected **into** an early-stage mouse embryo, to contribute to the development of all tissue lineages, including germ **cells**, in the resulting newborn mouse.

Whereas embryonic **stem** **cells** generally pass all these tests, many iPSCs do not. Closer examination of the **cells** that fail has revealed that the viruses used to deliver the four key reprogramming genes **into** skin **cells** are often not properly shut off, and important genes in the **cells**' original DNA are not properly turned on, resulting in **cells** that have lost their skin **cell** identity without gaining a **pluripotent** identity. These partially reprogrammed **cells** therefore do not qualify as authentic **pluripotent** **cells**.

Ongoing studies of iPSCs that do pass all the pluripotency tests are aimed at pinpointing the differences that distinguish a "good" from a "bad" iPSC. Thorsten Schlaeger, George Daley and their colleagues at Harvard University, for example, recently identified a pattern of gene activity in skin **cells** undergoing the lengthy (about three weeks) process of changing their identity to that of **pluripotent** **cells**. The fluorescent markers displayed by these **cells** during the transition distinguished them from **cells** in the same colony that would not ultimately become iPSCs, and so this marker pattern could be used as an early indicator of successful conversion.

Because scientists cannot ethically perform the most stringent pluripotency test by injecting human iPSCs **into** human embryos, it is absolutely critical to ensure that human iPSCs fulfill all other criteria of pluripotency. These include the complete silencing of the potentially harmful viruses employed to deliver the reprogramming genes. Yamanaka's team members discovered, for example, that one third of the mice that they had generated by injecting iPSCs **into** developing mouse embryos later formed cancers as a consequence of residual retrovirus activity.

One of the main problems with using retroviruses as gene-delivery vehicles is that these kinds of viruses (HIV is one example) integrate themselves directly **into** the host **cell's** DNA strand, becoming a part of its genome. This ability allows the added genes to reside permanently and remain active in the host **cell**, but depending on where the virus inserts itself, it can cause DNA damage that sparks cancerous changes in the **cell**. In efforts to produce safer iPSCs, therefore, many labs have developed methods that avoid permanent genetic manipulation of **cells**.

My research group has used a modified type of adenovirus, which normally causes the common cold in humans, to deliver the four reprogramming genes **into** mouse **cells** without integrating **into** the cellular genome. Adenoviruses persist inside the **cells** for only a short period--just long enough to convert them **into** iPSCs. When we injected the resulting **pluripotent** **cells** **into** mouse embryos, they readily became incorporated **into** the developing animals, which were all tumor-free as adults. This discovery, along with several alternative approaches to producing virus-free iPSCs, should eliminate a major roadblock to one day applying iPSCs directly in human therapies.

Ultimately, researchers hope to produce iPSCs without using any type of virus, but instead by simply exposing adult **cells** to a combination of drugs that mimic the effect of the re-programming genes. Sheng Ding of the Scripps Research Institute, Douglas A. Melton of Harvard and others have already identified chemicals that can substitute for each of the four re-programming genes in that each chemical activates a pathway of molecular interactions inside a **cell** that would be activated by the gene. When the four drugs have been tried together, however, they proved insufficient to make **pluripotent** **cells**. It may only be a matter of time, though, until researchers find the right cocktail and concentration of drugs to reprogram body **cells** **into** iPSCs without ever using viruses.

**Healing Cells?**

Because **pluripotent** **cells** are capable of generating any type of tissue in the body, the application that most captures the public imagination is the possibility of using iPSCs to produce replacement parts for **cells** and organs damaged by disease: neurons lost to Parkinson's or a spinal cord injury, for instance, or cardiac tissue destroyed by a heart attack. The ability to convert adult **cells** from the intended recipient of such a transplant **into** **pluripotent** **cells** and then coax those **cells** **into** the desired tissue would mean the replacement part is perfectly matched, genetically and immunologically, with the recipient's body. Moreover, easily accessible skin **cells** could be used to produce any kind of needed **cell**, including those in hard-to-reach organs and tissues, such as the brain or pancreas.

This technique also offers the possibility of repairing disease-causing genetic mutations before reintroducing the new **cells**, an approach that has been used with the adult **stem** **cells** that naturally regenerate some tissues. Success has been limited, though, because those precursor **cells** are notoriously difficult to grow and manipulate outside the body.

Recent experiments in mice suggest that treating genetic disorders in this manner with iPSCs is indeed feasible. Specifically, Rudolf Jaenisch of the Massachusetts Institute of Technology showed in 2007 that iPSCs could cure sickle **cell** anemia in an animal. The disease results from a single genetic mutation that causes red blood **cells** to adopt a deformed crescentlike shape. In this proof-of-concept study, investigators first reprogrammed skin **cells** from the mice **into** iPSCs. They then replaced the disease-causing gene in the iPSCs with a healthy version and coaxed the "repaired" iPSCs **into** becoming blood-forming **stem** **cells**. After transplantation back **into** the anemic mice, the healthy precursors produced normal red blood **cells**. In principle, this method could be applied to any other disease in humans for which the underlying gene mutation is known.

The multimillion-dollar question is how long it might take before iPSCs can be used to treat people. For the reasons already outlined, safety and control are absolutely essential before any iPSC-derived **cells** could be tested in humans. Current strategies to push embryonic **stem** **cells** or iPSCs **into** fully differentiated mature **cell** types cannot yet efficiently eliminate the occasional immature **stem** **cells** that might seed a tumor. An example underscoring why this is such a problem comes from a recent experiment in transplanting iPSC-derived dopamine-making neurons, which are the **cells** lost in Parkinson's patients, **into** rats suffering a version of the human disease. Although the rats clearly benefited from the engrafted **cells**, some of the animals also eventually developed teratomas in their brain.

In light of the fast pace of discoveries so far, however, it is optimistic but not unreasonable to estimate that such obstacles could be overcome in as little as 10 years, and transplantation of iPSC-derived **cells** might then be ready for human testing to begin. But iPSCs could well demonstrate their therapeutic value much sooner. The study and treatment of many tissue-destroying diseases, such as type 1 diabetes, Alzheimer's and Parkinson's, are limited by scientists' ability to obtain the affected tissues for study or to grow them in cultures for extended periods, and iPSCs could therefore be of enormous service in so-called disease modeling.

The idea is to derive iPSCs from affected patients' skin or blood **cells** and then convert them **into** the **cell** types involved in the patients' diseases. Both Clive N. Svendsen of the University of Wisconsin-Madison and Lorenz Studer of the Sloan-Kettering Institute recently derived iPSCs from the **cells** of patients with the devastating disorders smooth muscular atrophy and familial dysautonomia, respectively. When the iPSCs were transformed **into** the **cell** types affected in each of those diseases, the cultured **cells** recapitulated the abnormalities just as they are seen in patients.

This process could allow researchers to study the development of a disease in a petri dish, with the advantage of having a potentially endless supply of new **cells**, because the original iPSCs can be maintained indefinitely. Ultimately, the goal of academic scientists as well as pharmaceutical companies is to use these petri dish models to better understand the disease process and identify novel drugs to treat the illness.

This extremely promising use of iPSCs is not far off at all. Indeed, when Svendsen and Studer exposed their **cell** cultures to experimental drugs in each study, the disease "symptoms" were partially alleviated in the **cells**. This principle can now be applied to many other disorders for which treatments do not yet exist, and unlike transplanting **cells** **into** individuals, the result may be the development of drugs from which millions could benefit.

**Challenges and Hope**

Although iPSCs clearly circumvent some of the ethical and legal controversies surrounding embryonic **cells**, their pluripotency has yet to be completely understood or controlled, and embryonic **stem** **cells** therefore remain the gold standard for any **pluripotent** **cell** type.

Important unanswered questions include the practical issue of whether the conversion of body **cells** **into** iPSCs and the conversion of iPSCs **into** therapeutically relevant **cell** types can ever be made efficient enough for widespread use. Also unresolved is whether iPSCs retain any memory of the body **cell** type from which they are derived, a factor that could limit their ability to be converted **into** any other type of **cell**. We have gained some insight **into** the mechanisms by which a mature **cell** transforms **into** a **pluripotent** **cell**, but the process of reprogramming--how only a few genes manage to rewire the entire program of a mature **cell** **into** that of an embryonic **cell**--is still largely a black box.

Tackling such questions will require the continued use of embryonic **cells** as a reference point and will determine whether embryonic **stem** **cells** may be more effective for certain types of applications and iPSCs for others. Moreover, as truly **pluripotent** **cells**, iPSCs may raise ethical issues similar to concerns over embryonic **cells** because, in theory at least, iPSCs could be used to generate human embryos [see box on opposite page].

Nevertheless, from a scientific standpoint progress in the field of cellular reprogramming in recent years is truly astounding. Advances in cloning and, more recently, the discovery of iPSCs have refuted the old dogma that the identity of **cells** is irreversibly locked once they have differentiated. Both techniques have raised the possibility, at least, of reprogramming the identity of a body **cell** from one type of tissue **into** that of any other tissue type just by manipulating a few genetic switches. Understanding how this rewiring works at a mechanistic level will keep researchers energized and busy for years to come.

Only time can reveal whether iPSCs or related technologies will indeed become the modern Fountain of Youth. I personally think there is a good chance they will. Certainly iPSCs will continue to influence approaches to the study and treatment of many devastating diseases and have the **potential** to revolutionize medicine in the 21st century as profoundly as vaccines and antibiotics did in the 20th century.

**A Biological Clock**

**BASICS**

In the developing human body, a **cell's** possible identities become restricted with time and increased specialization--although **induced** **pluripotent** **stem** **cells** (iPSCs) seem to break those rules. Normally only the **cells** of an early embryo are **pluripotent**: able to become any **cell** type in the adult body. Later, embryo **cells** commit to lineages that limit their **potential** fates to specific tissue families, making them multipotent. In the adult body, **stem** **cells** are still more specialized. Mature body **cells** are said to be terminally differentiated--locked **into** their identities. Reprogramming rewinds the internal clock of mature body **cells** to a **pluripotent** state.

**CELLULAR POTENTIAL**

**Pluripotent**: Can give rise to any **cell** type

Multipotent: Can give rise to **cells** within a tissue family

Terminally differentiated: Locked **into** one identity

**Rapid Progress toward Safe Cell Rejuvenation**

**MILESTONES**

Just four years ago scientists in Japan first showed that a set of genes ferried by a retrovirus could transform the skin **cells** of adult mice **into** **pluripotent** **stem** **cells**. Many researchers have since been working to achieve the same end in simpler, safer and more efficient ways--key steps to making therapy a reality.

**2006** Shinya Yamanaka inserts four genes normally active in embryos **into** a modified retrovirus, which he then injects **into** mouse skin **cells**. The virus inserts the genes **into** the mouse DNA, and the genes then begin reprogramming the skin **cells** **into** **induced** **pluripotent** **stem** **cells** (iPSCs).

**2007-2008** Other researchers reproduce Yamanaka's accomplishment in mouse and human **cells**. Experiments also show that delivery of the four reprogramming genes by viruses that do not permanently integrate **into** cellular DNA still succeeds in producing iPSCs.

**2008-2009** Scientists demonstrate that iPSCs can be made using retroviruses carrying only three of the original Yamanaka reprogramming genes, then only two, or just by introducing the proteins encoded by the four reprogramming genes directly **into** **cells**.

**2009-2010** Scientists focus on raising efficiency by identifying distinct patterns of gene activation (revealed by fluorescent markers) characterizing **cells** that will successfully convert to iPSCs. Skin **cell** identity and reprogramming-gene markers give way to pluripotency markers.

**Custom-Tailored Cells to Cure Disease**

**THERAPY POSSIBILITIES**

An ability to transform a patient's skin or blood **cells** **into** iPSCs and then **into** any other type of **cell** could cure diseases in two ways: in the very near future, by allowing scientists to "model" illnesses and test drugs in a petri dish and, perhaps in another decade, by repairing or replacing diseased tissues.

**APPLICATION**

**DISEASE MODELING** Convert iPSCs derived from patients **into** the affected tissue type, then study disease progression and drug responses in those **cells**

**STATUS**

* Human iPSCs have already been used to generate 12 tissue types, including **cells** representing diverse disorders such as Parkinson's disease and diabetes
* Symptoms of smooth muscular atrophy and familial dysautonomia have been "treated" in cultured **cells**

**APPLICATION**

**CELL THERAPY** Convert iPSCs derived from a sick patient **into** healthy **cells** for transplantation **into** that individual

**STATUS**

* 10 years or more in the future
* iPSC-derived neurons have been transplanted **into** rats to treat a version of Parkinson's
* iPSC-derived blood progenitor **cells** with corrected sickle **cell** anemia genes cured the disease in mice

**THERAPEUTIC PROMISE**

Neurons were generated from **induced** **pluripotent** **cells** that were made from the skin **cells** of patients with Parkinson's disease. With the ability to take a mature body **cell** and convert it to an embryonic state, then **into** any desired tissue type, scientists will be able to study how a variety of diseases arise, develop and test drugs that hinder the disease process and, eventually, produce healthy replacement tissues for use in treating illnesses.

**CLONING**

Transferring the nucleus of a mature **cell** **into** an egg is another method of reprogramming a person's adult DNA to an embryonic state. Attempts to derive embryonic **stem** **cells** from human-clone embryos have so far failed for unknown reasons.

**TESTING CELLS' TRUE POTENTIAL**

Gold-standard laboratory tests to determine whether **stem** **cells** are truly **pluripotent** aim to demonstrate that the **cells** can give rise to any tissue type in the body. When injected **into** an early mouse embryo, for example, fluorescently marked **pluripotent** **cells** should integrate throughout the body of the developing mouse (bright green). Finding alternative methods of verifying the pluripotency of human iPSCs is an important goal.

**ETHICS UNCLEAR**

Injecting iPSCs **into** a developing mouse embryo yields a chimeric animal that displays the presence of foreign **cells** in its mixed coat colors. The same technique could, in theory, create a chimeric human embryo; iPSCs could also theoretically generate sperm and eggs to produce a human embryo through traditional in vitro fertilization. The pluripotency of iPSCs thus could raise some of the same ethical issues as human embryo research.

**CELLS FOR SALE**

The first commercially marketed product made from human iPSCs, a heart **cell** line called iCell Cardiomyocytes, is intended for use by pharmaceutical companies to test the effects of **potential** heart drugs.

**KEY CONCEPTS**

* **Induced** **pluripotent** **stem** **cells** are mature body **cells** that have been made to change their identities and revert to an embryolike state--without the help of eggs or embryos.
* Rejuvenating the normal body **cells** of any individual--then converting them to any of the 220 human **cell** types--could yield new disease treatments and custom replacement tissues.
* Scientists are now working to understand how these **cells** are able to reverse their biological clocks and whether the newest kind of **stem** **cell** will prove as powerful as embryonic **cells**.
* --The Editors

**MORE TO EXPLORE**

Induction of **Pluripotent** **Stem** **Cells** from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Kazutoshi Takahasi and Shinya Yamanaka in **Cell**, Vol. 126, No. 4, pages 663-676. Published online August 10, 2006.

Epigenetic Reprogramming and **Induced** Pluripotency. Konrad Hochedlinger and Kathrin Plath in Development, Vol. 136, No. 4, pages 509-523; February 15, 2009.

**Induced** **Pluripotent** **Stem** **Cells** and Reprogramming: Seeing the Science through the Hype. Juan Carlos Izpisúa Belmonte, James Ellis, Konrad Hochedlinger and Shinya Yamanaka in Nature Reviews Genetics, Vol. 10, No. 12, pages 878-883. Published online October 27, 2009.

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DIAGRAM: A Biological Clock

DIAGRAM: Rapid Progress toward Safe **Cell** Rejuvenation

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By Konrad Hochedlinger

Konrad Hochedlinger is associate professor of **stem** **cell** and regenerative biology at Harvard University and a faculty member of the Harvard **Stem** **Cell** Institute and the Howard Hughes Medical Institute. In his laboratory at Massachusetts General Hospital, he works toward understanding the biology of **stem** **cells** and cellular reprogramming and their **potential** use in the treatment of disease. He is also a scientific adviser to iPierian, a biopharmaceutical company developing products based on **stem** **cells**.

**Correction**

Konrad Hochedlinger's "**Your** **Inner** **Healers**" has two references to "smooth muscular atrophy"; it should have said "spinal muscular atrophy."

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