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INDUCED PLURIPOTENT STEM CELLS: A NEW HOPE OR A NEW CONTROVERSY?

By Sayandip Mukherjee

It seems unbelievable but it is true – cells within an organism are genetically almost identical and yet they are as dissimilar structurally and functionally as the human mind can conceive. The neurons which conduct the nerve impulses, the villi which line the gastrointestinal tract, the retinal cells which are responsible for our vision, and the immune cells which act as the body's sentinel have hardly anything in common between them. All these highly specialized cells are said to be terminally differentiated - they have lost their ability to become something else. Therefore, it was heralded as a significant breakthrough in science, when more than a decade ago, Ian Wilmut and colleagues at the Roslin Institute in Scotland demonstrated that a mature differentiated somatic cell taken from an adult mammal could be reprogrammed to give rise to an entire animal, which came to be known to the entire world as Dolly the sheep (Campbell 1996, 64).

The creation of Dolly using a technique known as somatic cell nuclear transfer (SCNT) proved beyond doubt that differentiation into adult cells does not imply an irreversible genetic fate for the cells, but that with the right manipulation these cells could be returned to their previous undifferentiated state, with all the potency to give rise to an entire animal (Figure 1). So, how can this knowledge be employed for the treatment of human diseases? The question leads us into the fascinating world of therapeutic cloning using stem cells.

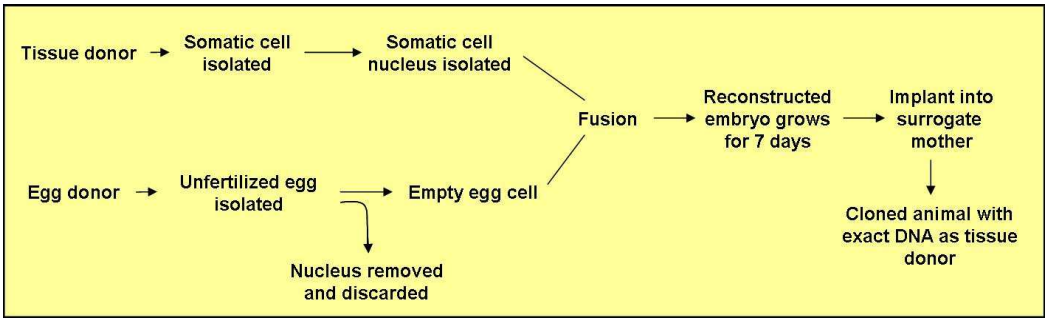

Figure 1. Schematic representation of somatic cell nuclear transfer (SCNT) technology. For therapeutic cloning, the embryo is used for isolating stem cells instead of being implanted.

Therapeutic cloning and stem cells

In therapeutic cloning (also referred to as non-reproductive cloning), following somatic cell nuclear transfer, the embryo develops into a multicellular blastocyst containing a layer of cells known as the inner cell mass (ICM) (Lanza 1999, 975). The ICM serves as a rich source of pluripotent embryonic stem (ES) cells (Figure 2). Another type of stem cell which is present in mature adult tissue is known as an adult stem cell. In the developing embryo, the ES cells can give rise to any cell inherent to the three germinal layers (ectoderm, endoderm and mesoderm), while the adult stem cells are primarily multipotent in nature and can only generate cell types of the organ from which it is derived: adult neural stem cells generate new nerve cells in the brain; bone marrow stem cells generate new blood and immune cells. Adult stem cells are generally concerned with repair, replenishment and maintenance of the organ systems.

Stem cells, by their definition, must possess two attributes: 1. the ability for *self-renewal* through mitotic divisions without undergoing any differentiation; and 2. the ability to differentiate into mature cell types which are referred to as totipotency, pluripotency or multipotency. Both adult and embryonic stem cells can be induced to undergo differentiation

into specific types of cells which can be used to treat disease conditions resulting from a defect concerning that particular cell type. As an example, stem cells can be induced to differentiate into myocardiocytes which can be used to treat the disease condition associated with heart muscle damage. The list of diseases which could potentially benefit from using ES cells is growing every day and presently includes Parkinson's disease and other neurodegenerative disorders, various forms of cancer, spinal cord injuries, and as mentioned previously, muscle damage disorders.

It is also noteworthy that pluripotent ES cells can be used for gene therapeutic approaches, whereby a defective gene can be substituted by a normal copy of the gene using an appropriate vector system. Furthermore, a significant advantage of using ES cells generated by the SCNT technology is that since the nuclear material is derived from the patient, the differentiated cells are considered 'self' by the body's immune system when transplanted back to the patient, and therefore there is less fear of tissue rejection associated with other forms of heterologous transplants.

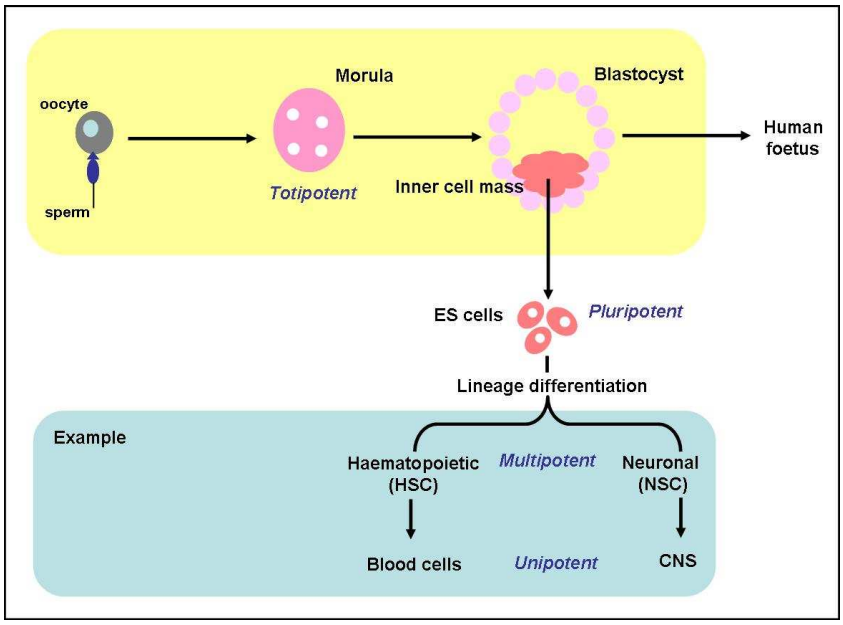

Figure 2. The origin of stem cells and their potency

The controversy

In spite of all its potential, the use of embryonic stem cells for therapeutic cloning has floundered since it was first proposed in the late 1990s, chiefly because of the unexpectedly difficult challenge of acquiring the human eggs necessary for the procedure. Considerable controversy has also been generated regarding the ethics of creation, usage and destruction of human ES cells for research purposes. Scientists who are in favour of stem cell research put forward the following arguments:

a. The benefits of stem cell research outweigh the costs in terms of embryonic life.

b. Embryos are not equivalent to human life since they are incapable of existing outside the womb. At this stage of their development they have no brain, no central nervous system, no pain receptors, no sensory perception and are fully devoid of any kind of consciousness.

c. In vitro fertilization (IVF) in fertility clinics produces a large number of unused embryos which are slated for destruction anyway. It will be worth it to utilise these unused embryos for research.

d. ES cells are far more superior in comparison to adult stem cells in that human embryonic stem cells are able to give rise to cells found in all tissues of the embryo except for the germ cells, while the adult stem cells are only multipotent in nature, being restricted to specific subpopulations of organ cell types.

On the other hand, pro-life activists and opponents to ES cell research argue that an embryo should be valued highly as a human life, since 'the line at which an embryo becomes a human life remains as arbitrary as ever' (Spallone 1989). Scientists who favour adult stem cell research think that there is an inherent scientific flaw in using ES cells since even these could be rejected by the immune system when transplanted back. Also, the employment of ES cells for treating diseases could be fatal in the absence of a highly-specific screening protocol; the presence of even one undifferentiated pluripotent stem cell in a culture of differentiated cells ready for introduction into the patient involves a potential risk of a tumour developing in the patient. In August 2000, The U.S. National Institutes of Health's Guidelines stated:

> […] Research involving human pluripotent stem cells...promises new treatments and possible cures for many debilitating diseases and injuries, including Parkinson's disease, diabetes, heart disease, multiple sclerosis, burns and spinal cord injuries. The NIH believes the potential medical benefits of human pluripotent stem cell technology are compelling and worthy of pursuit in accordance with appropriate ethical standards.

In this milieu of raging controversies, Shiniya Yamanaka and colleagues published results in 2006, based on a stunning experiment that seemingly offered to put an end to all the controversies surrounding the generation of ES cells for therapeutic cloning.

Induced Pluripotent Stem (iPS) cells

In the study conducted at Kyoto University, Yamanaka and his co-workers sought a core set of factors that would initiate the reprogramming of mature mouse cells into an embryonic-like state (Takahashi and Yamanaka 2006, 663; Takahashi 2007, 861). Using a selection scheme, they narrowed down from the initial 24 to the 4 key transcription factor genes essential for pluripotency in ES cells. They introduced these 4 genes (*c-Myc*, *Oct3/4*, *Sox2* and *Klf4*) simultaneously into mouse embryonic fibroblasts using genetically modified retroviruses and observed that some of these fibroblasts were transformed into ES cell-like cells (Figure 3).

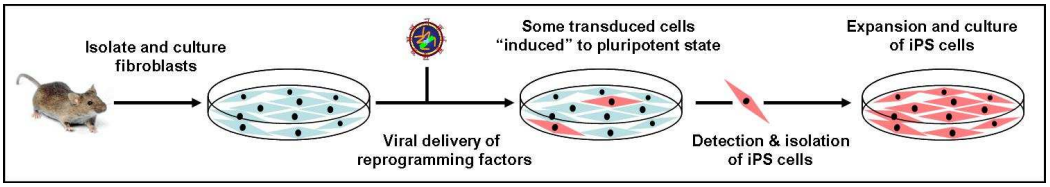

Figure 3. Schematic representation of generation of iPS cells from adult mouse fibroblasts.

The 'unique selling proposition' of Yamanaka's protocol lies in the simplicity of the genetic trick – using only 4 transcription factors to reprogram a terminally differentiated cell into a pluripotent entity which they now called induced pluripotent stem (iPS) cells. Yamanaka's group also demonstrated that iPS cells can be derived from both adult and fetal human fibroblasts. Taking cue from the fact that the chimeric mouse derived from iPS cells developed tumours (most likely

due to the expression of c-Myc), James Thomson's group from Wisconsin Madison omitted *c-Myc* from the reprogramming cocktail and were still able to reprogram fetal and neonatal human fibroblasts (Yu 2007, 1917). *Oct3/4* and *Sox2* were common to both of the strategies.

The iPS strategy also broke down the technical barrier that had been hindering the progress of therapeutic cloning. Apart from the requirement of standard tissue culture set-up and a small scale laboratory effort involving the knowledge of molecular cloning, almost no special techniques, animals, eggs or embryos are necessary. This is in sharp contrast to the generation of ES cells, whether starting from left-over peri-implantation embryos or using the SCNT, both of which require high technical skill and an expensive array of equipments.

Excited by these findings, more and more researchers are jumping onto the iPS bandwagon. Policy makers, pro-life activists and some scientists have also started arguing that all research pertaining to the isolation of ES cells from embryos should be halted in favour of the iPS cell strategy. However, there are a few caveats that should be borne in mind (Cyranoski 2008, 406):

1. The very low efficiency (approximately 0.01%) of iPS cell generation from both adult and fetal fibroblasts presents a serious concern. However, since getting enough somatic cells is not a huge problem, this can be easily overcome. It is trickier to find these few reprogrammed cells and then culture them under suitable conditions to prevent them from differentiating into a particular cell lineage.

2. Myc-containing iPS cells were found to be clearly oncogenic since they gave rise to tumours. However, even after excluding Myc, the rest of the three reprogramming factors from Yamanaka's cocktail have oncogenic potential. Over-expression of the *Oct3/4* gene has been observed in bladder cancer, *Sox2* has been implicated in various forms of carcinomas (pancreatic, stomach, breast, brain), and the *Klf4* gene is often over-expressed in squamous cell carcinoma.

3. The current protocols for iPS cell generation also rely on the use of retroviruses which integrate randomly into the human genome and can cause insertional mutagenesis. This risk is well-established from clinical trials involving gene therapy for X-linked severe combined immunodeficiency (X-SCID), and therefore should be seriously evaluated.

4. A major question raised by the doubters of this technology is whether the iPS cells will differentiate as stably and diversely as the ES cells. Although the similarities between the two have been established in terms of markers of pluripotency, the results are variable from group to group.

5. Inconsistent with the transcriptional logic of nuclear programming, the factors were found to be unable to reprogram more developmentally matured somatic cells such as neonatal foreskin fibroblasts, adult mesenchymal stem cells and dermal fibroblasts.

How programmable is the reprogramming?

The origin of iPS cells from amongst the somatic cells has not been validated fully by any of the groups at the forefront of the technology (Liu 2008, 391). Though it has been dismissed by Yamanaka's group, it is theoretically possible that the iPS cells originate from the rare stem cells co-existing in mouse embryonic fibroblast culture. This possibility gains further strength on the basis of the observation that the induced cells are heterogeneous in nature, quite unlike the ES cells which have homogenous qualities. Also, in spite of the widespread acceptance of iPS cell strategy, it is not yet understood whether the 'fantastic four' truly represent a core regulatory reprogramming circuit. Myc, which was an essential part of Yamanaka's initial 'cocktail', was later found to be dispensable. Yu et al. used a different set of factors (Oct4, Sox2, Nanog and Lin28)

to induce pluripotency in human somatic cells, thereby showing that even Klf4 is not a necessary ingredient in the cocktail. All these observations quite pertinently bring up the question as to what role these factors really play in the entire process. How can different sets of genes have the same outcome? Is it possible that these genes might play a very small role in switching on more widespread downstream genetic cascades that are yet to be discovered? It should also be noted that the triggering of the change for reprogramming the somatic cells must be silenced at some point during the generation of iPS cells. Therefore, it is also necessary to investigate how the involved genes set up the condition in which the epigenetic state of the cell is reprogrammed.

The Application of iPS cells: Promises and Pitfalls

Although a lot of research needs to be performed before iPS cells can be considered as a safe and efficient alternative to ES cells and the SCNT strategy, promising results vis-à-vis proof-of-concept is already available. Rudolf Jaenisch's group at MIT derived haematopoietic progenitor cells from mouse iPS and successfully treated mice suffering from a humanized version of sickle cell anaemia (Hanna 2007, 1920). Jun Takahashi's group at Kyoto University is pursuing clinical treatment of a monkey model of Parkinson's disease with neuronal precursor cells derived from ES cells as well as iPS cells.

In the near future, iPS cells could provide researchers the tool to culture a 'disease in a dish'. Individuals with known susceptibility to genetic diseases could provide starting materials for generating iPS cells that could be used for the study of pathogenesis and for the development of new therapies (drug screening protocols). Building large banks of pluripotent cells of desired human leukocyte antigen (HLA) haplotypes for tissue matching would be made possible by employing the iPS cell strategy without sacrificing hundreds of embryos, thereby bypassing ethical concerns (HLA haplotypes are unique genetic fingerprints which are inherited from parents). Differentiated cells from haplotype-matched iPS could be used for treating patients without the fear of immune rejection.

The scientific world will be watching with great interest the first clinical trial of embryonic stem-cell-based treatment to be conducted by the California-based pharmaceutical company, Geron, in the middle of 2008. They will implant oligodendrocytes derived from stem cells of patients with spinal-cord injuries. Results from this trial could potentially decide the readiness with which iPS cells generated from human patients would make their way into the clinic. Before that, however, it will be important to weed out any leftover undifferentiated iPS from cultures of differentiated cells since the presence of a single pluripotent iPS cell could be potentially tumourigenic.

Conclusions

The reprogramming studies of Wilmut in 1997 and of Yamanaka in 2006 addressed the same question: What is a cellular state? So far, we have assumed that a cell undergoing differentiation goes through distinct and defined steps to reach the terminal differentiation step carefully orchestrated by specific transcription and epigenetic factors. Contrary to this notion, SCNT technology and the iPS cell strategy have conclusively shown that it might not be such a linear, one-directional occurrence (totipotency >pluripotency > multipotency > unipotency), but instead, it is possible for the cell to 'jump' from one state of potency to another, completely bypassing the intermediate steps. A detailed understanding of what determines a cell's epigenetic state might even make it possible in the future to reprogram one cell type as another without reverting back to the pluripotent stage. The discovery of iPS cells was heralded as a significant milestone in cloning and stem cell research. Whatever the future has in store for this nascent field of human biology, the generation of iPS cells reinforces Daniel Dennett's remark in *Darwin's Dangerous Idea* that 'biology is not just like engineering; it is engineering'.

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