

## Stem Cells and Drug Discovery: the Time Has Never Been Better

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### Abstract:

*Human embryonic stem (ES) cells (hESCs) have been recognized as a valuable resource for advancing our knowledge of human development and biology, and for their great potential in regenerative medicine and drug discovery, and remain to be the gold standard for research on pluripotency and differentiation. Although hESCs hold great promise because of their unique ability to self-renew and their developmental potential to form various cell lineages in the body, traditional techniques for generating hESCs rely on surplus IVF embryos and are incompatible with the generation of genetically diverse, patient or disease specific stem cells. A recent breakthrough in stem cell biology is the success of converting human somatic cells into stem cell-like cells, termed induced pluripotent stem (iPS) cells (iPSCs), by using defined reprogramming factors. Human adult cells such as ordinary skin cells can now be induced to revert back to earlier stages of development and exhibit properties of authentic ES cells. While these reprogrammed cells have similar developmental potential as hESCs, they are not derived from human embryos. The iPS cell technology would truly prove very useful for the generation of individual cell lines from many different patients to study the nature and complexity of disease. Moreover, the problems of immune rejection for future therapeutic applications will be greatly relieved by being able to generate reprogrammed cells from individual patients. Recently, a flurry of research has yielded critical insight into the understanding of the technology, logic, safety, and utility of iPS cells. The development of the iPS cell technology has led to the use of these unusual cells for disease modeling, drug discovery and regenerative cures, paving the paths to new medicines.*

### Human embryonic stem (ES) cells (hESCs): Still the gold standard as models for development and disease research and as platforms for drug discovery and toxic testing applications

Animal models have been invaluable for drug discovery and development. However, they are limited in their ability to predict the efficacy and toxicity of drugs in humans. Drugs tested to be efficacious in animals do not always translate to the same efficacy in humans. Certain chemicals predicted as "safe" from animal studies induce toxic outcomes in humans. The most striking illustration of this limitation is thalidomide, which did not adversely affect rodent development and was falsely believed to be safe for humans. Gestational thalidomide exposure caused severe limb defects in 20-30% of exposed children. Thus, the general limitation of animal models to elucidate human response should not be taken lightly. A new generation of models with greater predictive ability and mechanistic insight into human development and function is strongly warranted.

Differentiated cells or tissues derived from multipotent or pluripotent stem cells offer the potential to address the critical shortage of human cells and tissues available for drug efficacy

and toxicity evaluation and assessment. Stem cells are specialized cells that not only maintain the capacity for self-renewal, proliferating without further differentiation, but also possess the ability to generate progeny that can differentiate down multiple developmental fates. Somatic stem cells from tissues such as brain generally have a limited repertoire of developmental choices and are often called multipotent, while stem cells from early embryonic stages have broader developmental potential.

Human embryonic stem (ES) cells (hESCs) isolated from the inner cell mass of human blastocyst embryos have the potential to differentiate into all of the cell types in the human body, and are thus defined as pluripotent. ESCs are capable of both self-renewal and differentiation. Pluripotent hESCs can give rise to various differentiated cell types in the body. A critical feature of hESC-derived cell types and their ability to reveal mechanisms underlying human development and disease is that these cells function as *in vivo* counterparts. For example, it has been shown that the duration of neuronal and glial differentiation from hESC-derived neural precursors is similar to lineage allocation *in vivo*, and they undergo key events of morphogenesis including embryoid body formation and neurogenesis. The value of ES cells to predict if and how drugs work and cause toxicity or disrupt development has been validated by an increasing body of evidence.

For ethical reasons, experimental drugs and toxic agents can not be directly tested on humans and especially in human embryos. The pluripotency of hESCs thus offers a useful surrogate to examine drug efficacy and to evaluate toxic risk. A broad range of genetic tools are available for their study and manipulation, and reporter lines can be generated for use in high throughput screens for drug efficacy. The use of hESCs in developmental toxicity testing should refine our understanding of molecular mechanisms, producing more sensitive and specific endpoints or biomarkers for toxicity evaluation and detection of adverse effects in humans. The use of genetically diverse hESC lines in biomarker discovery will also provide invaluable insight into the relationship between genetic factors and drug efficacy or toxicity. However, faithful recapitulation of whole organs has not been demonstrated by differentiation of hESCs. Thus, despite producing functional human cell types, their ability to wholly mimic human development is incomplete. This limitation will be better addressed through the integration of hESC technology to humanized mouse models of developmental biology and toxicity.

The use of human ES cells remains controversial and

poses ethical considerations since current isolation techniques result in the destruction of the embryos from which the cells were obtained. Recent advances in stem cell research have led to the development of methods to “reprogram” differentiated somatic cells from mice and humans toward an undifferentiated state that closely resembles that of ES cells. The de-differentiated cells have been termed induced pluripotent stem (iPS) cells (iPSCs) because, like ES cells, they are able to differentiate into cell types representing all three embryonic germ layers. Although these recent findings demonstrate the feasibility of developing pluripotent stem cells from non-embryonic sources, the generation of these iPS cell lines was accomplished by infection with multiple retroviral vectors encoding transcription factors. The use of viral vectors presents challenges that are likely to complicate or preclude the full range of potential applications of this approach for generation of difficult to obtain human cell types for use in drug efficacy or toxicity testing. Methods to derive iPS cells without using viral vectors would obviate these concerns and expedite development of drug efficacy and toxicity testing applications.

In addition, multipotent somatic stem cells have been identified in a number of human tissues including the brain. Each type of somatic stem cell is capable of differentiating into a limited number of specific cell types that make up the tissue of origin. For example, neural stem cells can differentiate into neurons, astrocytes, and oligodendrocytes. Although some reports indicate that somatic stem cells may have the ability to differentiate into cells of other lineages, others suggest that this apparent trans-differentiation could be due to fusion with other cell types. The true extent of somatic stem cell plasticity and the potential utility of trans-differentiated cell types in human toxicity assays have yet to be determined. However, the difficulty in obtaining a pure population of somatic stem cells and once acquired, expanding these cells to sufficient quantities while maintaining the progenitor state remains significant obstacles to their use as cell source for human therapy and toxicity testing applications.

### **Induced pluripotent stem (iPS) cells (iPSCs): Paths to new medicines**

The process of directly turning one cell type into another, termed cellular reprogramming, has opened up a new field of biology and holds out hope of life-saving medical advances. In particular, the recent remarkable success of reprogramming differentiated cells such as fibroblasts back to a pluripotent state, similar to that of embryonic stem cells, demonstrates the feasibility of turning back the

clock of somatic cells to create induced pluripotent stem (iPS) cells (iPSCs) - stem cells made without the use of embryos<sup>[1-6]</sup>. The iPS cell technology is becoming a major tool for modeling disease and drug discovery. Moreover, if cellular reprogramming will be made more efficient and safer, patients may someday be treated with healthy versions of their own cells, avoiding immune rejection in therapy. This article seeks to address the four P's (promise, progress, pitfalls, and potential) of iPS cells in a broad context of the logic, utility, and safety of cell reprogramming as well as implications for therapeutic use.

The iPS cell is truly an amazing story. Although Shinya Yamanaka's invention of iPS cells in 2006[1] was far from serendipity, I suspect that even the man, who started it all to create this field himself, would admit sometimes a little "luck" might be helpful in the pursuit of groundbreaking science. Throughout this article, I discuss the "LUCK" of iPS cells: the *Logic, Utility, Concern*, and "King of all cells" property (i.e., pluripotency) of these unusual cells.

### **Capitalizing on the promise of iPS cells: A catalyst for disease modeling, drug discovery, and regenerative therapy**

The remarkable developmental potential of pluripotent stem cell research promises to introduce a new era of science and medicine. The defining features of pluripotent stem cells – their ability to self-renew and their ability to differentiate into virtually all of the specialized cells of the body – have given rise to the compelling dream of being able to restore function after disease or injury by replacing damaged cells with healthy new cells. Stem cells may provide the cellular components for the even more ambitious task of reconstructing entire tissues someday, and offer a powerful tool for understanding a variety of diseases such as cancer, Alzheimer's disease, Parkinson's disease, autism, and multiple sclerosis, so that targets for new therapeutics can be defined.

Human ES cells hold great potential in regenerative medicine and drug discovery. However, one major problem for hESC-based therapy is that the cells derived from hESCs will be rejected by the recipient and can only be tolerated under persistent immunosuppression, which itself can cause cancer and infection. The development of iPS cells, which are generated from somatic cells of individual patient with defined factors and very similar to hESCs, could provide ideal cell source for transplantation by avoiding graft rejection in the patient. In addition, the disease-specific iPSCs can be

used as human disease models for more reliable testing of the efficacy and toxicity of drugs. However, there are several major bottlenecks that prevent the development of iPSCs in human therapy and drug discovery. A pressing goal of iPS cell research is to resolve the major bottlenecks remained in human iPSC biology to make it feasible for human therapy and drug discovery. We need to develop safe and efficient approaches to generate iPSCs from human patients. We need to develop strategies to eliminate the risk of teratomas associated with the undifferentiated iPSCs. We need to develop mouse model with functional human immune system to study the immune responses and tolerance during transplantation. Resolving these bottlenecks will greatly facilitate the development of the iPS cell technology into stem cell therapy and disease models for drug discovery.

### **Sweating the technological improvements: Towards safer, efficient iPS cell generation**

Yamanaka group first described the identification of the combination of four transcription factors whose retroviral overexpression enabled the induction of a pluripotent state in murine<sup>[1]</sup> and human<sup>[2]</sup> fibroblasts. The remarkable finding was that simultaneous overexpression of Oct4, Sox2, and Klf4 with<sup>[1,2]</sup> or without c-Myc<sup>[5]</sup> led to generation of iPS cells that were similar to ES cells. Thomson group also reported similar success in *in vitro* reprogramming of human fibroblasts with lentiviral overexpression of another combination of reprogramming factors (Oct4, Sox2, Nanog, and Lin28)<sup>[3]</sup>. Reprogramming of mouse and human somatic cells was made 10-fold more efficient by using a single polycistronic vector carrying the reprogramming factors<sup>[7]</sup>. To address cancer risk and other safety concerns, several approaches have been devised to generate iPSCs free of the exogenous reprogramming factor genes, including the use of non-integrating adenoviral approaches for transgene delivery<sup>[8,9]</sup>. The piggyBac transposon system was used to introduce reprogramming genes and to induce pluripotency and then to remove the transgenes from established iPSC lines<sup>[10,11]</sup>. The Cre/loxP recombination system was used to excise exogenous reprogramming genes from human iPSCs after the cells are reprogrammed<sup>[12]</sup>. Yu et al. demonstrated the successful use of non-integrating "episomal" vector to obtain iPSCs free of vector and transgenes<sup>[13]</sup>. All of the above-mentioned iPS cell methods were based on the use of DNA material. Reprogramming protein-based methods were then successfully developed and implemented<sup>[14,15]</sup> to overcome additional iPSC safety concerns. To date, there have not been reports of purely chemical-induced

iPS cell generation. However, much success has been made in enhancing efficiency and success rate of DNA-based or protein-based methods of iPS cell generation by adding chemicals onto the protocols<sup>[16,17]</sup>. In addition, attempts are under way to conduct large scale chemical library screening in order to identify additional chemicals capable of inducing pluripotency<sup>[16,17]</sup>.

### **The technology: Towards a holistic approach of cellular reprogramming?**

Thus far, various iPS cell technologies have been DNA-based, protein-based, and/or chemical-based, using defined factors, i.e. specific genes, proteins, and/or chemicals. There have not been reports of an RNA-based protocol of iPS cell generation. A recent paper<sup>[18]</sup> described an RNA-based method termed “transcriptome induced phenotype remodeling (TIPeR)” for cellular phenotype transfer/conversion, and may represent a new, seemingly “holistic” approach of cellular reprogramming. The logic of this RNA-based cellular phenotype conversion may be applicable to iPS cell generation. This approach relies on the transfer of the “entire” regulatory components from a target cell to a donor cell, enabling cellular reprogramming by manipulating “whole systems”, rather than by a small set of master genes. Laser light induced phototransfection was used to transiently produce pores in the host cell, through which RNA species could diffuse. The results showed that donor cell RNA species could induce long-term changes in genomic transcription of the host cells, thereby changing the functional phenotype of the host cells to that of the destination phenotype. The results also showed that RNA transfer triggered molecular state changes in the differentiated host cell. Such changes involved reprogramming of the host cell’s pattern of gene expression, leading it to take on a distinct destination cell phenotype, which would resemble the phenotype of the differentiated donor cell. Thus, the destination phenotype in the recipient cell is generated by phototransfecting mixtures of RNA species directly isolated from donor cells, and the phenotype conversion is due to the activity and abundances of the specific proteins made from the host cell RNA mixture.

The logic of cellular phenotype transfer/conversion may be applicable to cellular reprogramming of other lineages or to iPS cell generation. An understanding of the logic of cellular phenotype conversion may permit the creation of specific cell types at will. Unlike DNA-dependent expression systems, where just a few introduced nucleic acid species are expressed, the RNA-based methodology permits the simultaneous translation of the en-

tire pool of the introduced RNA species. This approach yields an expression system that closely mirrors specific cellular environments and that is free of overexpression artifacts. Thus, in light of the results from this somewhat “benign” approach of cellular reprogramming, it may be reasonable and timely here and now to anticipate and work towards a “holistic” approach for safe and efficient iPS cell generation.

### **The logic of genomic reprogramming: Unraveling the “black box”**

To attempt to understand the logic of cellular reprogramming and to unravel the “black box”<sup>[19,20]</sup>, it may be useful to conceptualize each cell type as having reached a distinct molecular steady state through as-yet-incompletely understood genome dynamics involving gene regulation, epigenetic modifications and molecular cell physiology. Cellular phenotype can be thought of as a continuum of states, and particular cell types are the locally stable states that are reached during cell differentiation.

What is the genomic logic that separates these different states? Are the states transformable in a live cell? Is it possible that differentiated cells are the phenotypic representations of molecular steady-states of a genome dynamical system, and is it possible that cells can be moved from one steady state to another through transcriptome manipulations? Having the ability to transfer cellular phenotype by manipulating “whole systems” would enable a new level of functional investigations of the cell, allowing for alteration of cellular identity in a systematic manner, rather than gene-by-gene.

To understand the sequence of changes in genome dynamics involved in cellular phenotypic reprogramming, we could measure various molecular correlations of the reprogramming event over time, and assess steady state levels of cellular RNA or proteins. This measurement would provide an unprecedented opportunity to develop a molecular map of genome dynamics in relation to cell phenotypes. This map will describe dynamic trajectories of molecular states between cell types and provide a unique foundation for distilling a genome dynamics model that will aid to decipher the underlying molecular logic regulating cellular phenotype and function. Such a dynamic molecular map, coupled to an enabling technology for manipulating transcriptome dynamics, has the potential to transform functional genomics with far-reaching applications, where any cell source can be

turned into a model system for tissue-specific processes and therapeutics.

### **The concern: Addressing the link between pluripotency and tumorigenicity**

Recently, a flurry of studies have revealed that key players involved in genome integrity and chromatin remodeling are also critical in stem cell pluripotency and reprogramming, thus further strengthening the fundamental link between tumorigenicity and pluripotency. Will iPS cells be safe? And what are the pitfalls of the iPS cell technology?

The accurate repair of DNA damage is crucial for the maintenance of genome integrity and the prevention of tumors. A pair of recent studies<sup>[21,22]</sup> have revealed a novel function of a gene product called “Amplified in Liver Cancer 1” (Alc1, also known as “Chromodomain helicase DNA binding protein 1-like”, Chd1L), which has been identified as a molecule that may indicate the onset of cancer. ALC1 belongs to the SNF2 gene family, and is found in excessive amounts in approximately 50% of liver cancers. The protein encoded by the Alc1 gene is a chromatin remodeling enzyme. The protein normally loosens tightly-packaged chromatin when DNA damage occurs in cells. Once chromatin is loosened, the damaged DNA is exposed to repair molecules. In cancer cells, however, too much ALC1 can overly relax the chromatin, making the DNA vulnerable to mistakes and thus raising the risk of tumorigenicity.

Alc1, a chromatin remodeling enzyme, is activated by another enzyme, poly(ADP-ribose) polymerase-1 (PARP-1), in a process termed “PARylation”, i.e. poly(ADP-ribosylation). PARylation is a posttranslational protein modification carried out by PAR polymerases. PARP-1 is the most abundant of 17 PARP family members, accounting for >85% of nuclear PARP activity. PARP-1 is a DNA nick sensor enzyme that is activated by DNA breaks. Activated PARP cleaves NAD into nicotinamide and ADP-ribose and covalently attaches a poly(ADP-ribose) (PAR) polymer to itself and other suitable protein substrates. The binding of a specific Alc1 region to PAR coupled to PARP-1 helps to recruit Alc1 to bind to and remodel nucleosomes<sup>[21,22]</sup>.

PARP-1 and PAR are well known to play critical roles in chromatin organization, transcriptional regulation, and DNA replication and repair, but the underlying mechanism is largely unclear. Finding that PARP-1 and PAR recruit the chromatin remodeler Alc1 to chromatin and activate Alc1 activity suggests a mechanism by which

they may function. Alc1 activities could be blocked by PARP-1 inhibitors<sup>[21,22]</sup>, which have already been extensively investigated for the treatment of cancer and other diseases, suggesting that the therapeutic activities of PARP-1 inhibitors could be due in part to indirect effects on Alc1. Thus, this finding identifies a new function of the SNF2 family member Alc1 (Chd1L) in cancer.

Interestingly, another SNF2 gene family member, Chd1, has recently been identified as critical for embryonic stem cell pluripotency<sup>[23]</sup>. Chd1 seems to act by keeping the chromatin open and thus poised to express genes for maintaining “stemness”. Chd1 was also shown to be crucial for reprogramming somatic cells back to a pluripotent state<sup>[23]</sup>. Thus, inhibition or depletion of Chd1 hinders the generation of iPS cells from adult cells<sup>[23]</sup>. Further studies regarding what other molecules work in concert with the Chd1 gene to direct chromatin remodeling may aid efforts to increase the efficiency and safety of iPS cell generation, and may also shed new light on how cells transition from one type to another, a process that happens normally during embryonic development and goes astray in cancer. Since other SNF2 gene family members including ACL1<sup>[21,22]</sup> are regulated by PARylation, it would surely be interesting to investigate whether PARP-1 and PAR are involved in chromatin remodeling by Chd1 and how the chromatin open state is maintained by Chd1.

Surprisingly, a recent study has shown PARP-1 and PAR may instead help to condense the chromatin during DNA damage<sup>[24]</sup>. Using a laser-induced DNA damage paradigm, the researchers demonstrated that PARP-1 was activated, and PAR was formed and was rapidly detected by a family of macrodomain proteins that were able to quickly move to the site of DNA damage in cells. A high-resolution image, obtained by X-ray crystallography, showed that the macrodomain forms a ‘pocket’ fitting the PAR signal exactly. Unexpectedly, among the members of PAR binding proteins found is a protein called histone macroH2A1.1<sup>[24]</sup>. Histones play a major role in assembling chromatin and keeping it together, but they do not usually have macrodomains. PARP-1 and macrodomains may together help to maintain a healthy genome in non-cancerous cells. Cancer cells, however, do not have macroH2A1.1. As the protein normally helps to rapidly detect DNA damage, macroH2A1.1 may be a “missing” link in cancer that could contribute to the disease. Because macroH2A1.1 is embedded in chromatin, when it recognizes PAR at DNA damage sites, it drags the complex but highly-organized tangle of

chromatin with it. As a result, macroH2A1.1 condenses the chromatin environment around the damaged area. It is possible that, by temporarily compacting the DNA, the broken ends of the DNA molecule are kept closer together, increasing the chances of being able to repair it. The precise role of PARP-1 and PAR in chromatin plasticity, as well as in stem cell pluripotency, however, remains to be addressed.

Recently, there was a tsunami of unprecedented simultaneous publications of five papers in *Nature*<sup>[25-29]</sup> on the topic of the tumor suppressor p53 as a barrier to induced pluripotency, and inactivating or deleting p53 allowed for a 100-fold increase in cell reprogramming efficiency. In addition, Banito et al.<sup>[30]</sup> showed that expression of the four reprogramming factors (Oct4, Sox2, Klf4, and c-Myc) triggered senescence by up-regulating p53, p16(INK4a), and p21(CIP1) due to induction of DNA damage response and chromatin remodeling of the INK4a/ARF locus, thus identifying senescence as a barrier to pluripotency.

PARP-1 and p53 are well known to both physically and functionally interact with each other. Together, PARP-1 and p53 earn the nickname “guardians of the genome”. A recent report<sup>[31]</sup> indicated that PARylation promotes embryonic stem cell differentiation. Thus, it is reasonable to speculate that PARP-1, like p53, may impede somatic cell reprogramming back to a pluripotent state. Clearly, although already well studied, many of the biological functions of the PARP-1/p53 complex still remain to be discovered. It may be useful and timely here and now to explore the role of this dynamic complex in ways toward more efficient iPS cell generation.

### **Breaking the barriers for more efficient iPS generation, but at what cost?**

Would all these new studies support a notion that molecules critically involved in genome stability, such as PARP-1 and p53, function as not only guardians of the genome, but also barriers to pluripotency? Genome integrity may indeed be fundamental in stem cell pluripotency and reprogramming. With the recent demonstration that iPS cells pass an ultimate test for pluripotency and in fact yield live mice<sup>[32-34]</sup>, all these findings further strengthen the fundamental link between induced pluripotency and tumorigenicity. It would appear as if the major guardians of the genome are roadblocks for iPS cell generation, and simultaneously and systematically destroying these roadblocks would truly improve reprogramming efficiency. But at what cost, and will it be

safe? Reprogramming methods coupled with inactivating or deleting p53 allowed for cells even with heavy DNA damage to be turned into stem cells<sup>[27,29]</sup>. Although the methods may not seem desirable at first (at least not for therapeutic use of iPS cells), they could help to establish useful cellular models for a variety of diseases in which somatic cells are difficult to reprogram. It is possible that cancer can develop when p53 activity is diminished. However, cells can be restored to their original state once iPS cells have been created, or transient p53 inactivation by chemicals or other means can be sought, in order to prevent from trading cancer risk for reprogramming efficiency. Since the efficiency of cell reprogramming technologies is woefully low, any methods that help to improve efficiency would be of practical uses.

Clearly, critical new insights gained from a flurry of recent investigations into key players involved in genome integrity, chromatin plasticity, and how to keep chromatin “loose” link tightly cancer and stem cell biology.

### **The Utility: Using the iPS technology for reverse engineering human disease**

The ability to de-differentiate patient-specific adult cells back to stem cells and then to re-differentiate them into specific lineages would allow to create appropriate in vitro models of “disease in a dish”. Such a “de- and re-differentiation” reprogramming approach is to make a “cellular U-turn”, and is ideal to track down the root causes of human disease. The iPS cell technology would prove useful for the generation of individual cell lines from many different patients to study the nature and complexity of disease. In addition, the problems of immune rejection for future therapeutic applications of this work will be greatly relieved by being able to generate reprogrammed cells from individual patients. This research truly represents an exciting new research direction that makes use of the iPS technology for reverse engineering human disease, and accomplishments through this line of research would undoubtedly have important implications for catalyzing progress in understanding pathogenesis and developing treatments for human disease.

Human ES cells hold great promise in regenerative medicine because of their unique ability to self-renew and their developmental potential to form various cell lineages in the body. Traditional techniques for generating hESCs rely on surplus IVF embryos and are incompatible with the generation of genetically diverse, patient or disease specific stem cells. With the iPS cell technology, ordinary adult human skin cells conveniently

collected by a simple skin punch biopsy can be induced to revert back to earlier stages of development and exhibit properties of authentic human ES cells. While these reprogrammed cells have similar developmental potential as hESCs, they are not derived from human embryos. The iPS cell technology would truly prove very useful for generating disease models and cell-based therapeutics.

For years, researchers have grown human cells in the laboratory in an attempt to mimic various diseases, but the available techniques had significant shortcomings. Cells taken directly from affected patients typically have a limited lifespan when grown in laboratory dishes, restricting the types of studies for which they can be used. Researchers often turn to cells that have been modified to make them live in a dish forever, but altering cells to make them immortal changes their physiology and can cast doubt on a study's results.

With the demonstration that adult cells can be converted to stem cells by introducing a set of genetic "reprogramming factors", it is now possible to produce disease-specific stem cells; the resulting stem cell cultures would harbor the disease genome of the donors. In many cases, these new stem cell cultures will mimic human disease more reliably than animal models. Despite the vast genetic similarities between humans and mice, physiological differences invariably affect the course of disease in a mouse. In some cases, the genetic defect that produces a disorder in humans does not cause the same symptoms in mice. Thus, human cell cultures are an essential complement to research with animal models. The most immediate application of the disease-specific stem cells will be to reproduce human diseases in culture to explore their development in different tissues. The technique will even enable researchers to compare how the same disease varies among people, by generating disease-specific stem cell cultures from many individuals. The cells will also offer a proving ground for screening drugs to treat disease and for testing drug toxicity. Over the longer term, the technique will be applied clinically towards cell-based therapy, and may eventually allow us to develop therapies using a patient's own cell - reengineering the cells to correct disease-causing defects then re-introducing them into the body.

### **The iPS technology: An exploratory tool**

The success of generating iPS cells by reprogramming human somatic cells back to a pluripotent state via defined factors is also an enabling technology and

an exploratory tool. This amazing technology will be a vehicle for many tantalizing possibilities of technological developments, and also represents a platform for a wide variety of lines of basic and applied biology investigations[35]. For example, the iPS cell method can be an innovative tool for human mitochondrial research, since we can generate iPS cells with normal or abnormal mitochondria from healthy people at different ages and patients with different mitochondrial genetic diseases, and then force the iPS cells to differentiate into various cell lineages to evaluate how the state of mitochondrial structure and function will dictate the ability of the iPS cells to provide distinct lineages. In addition, combining human iPS cells with mouse models would provide the microenvironmental milieu to support the tissue's physiological function within the context of the whole organism, enabling greater understanding of mitochondrial function *in vivo* and pathogenesis of human mitochondrial diseases. Transplanting human iPS cells into mice would thus create humanized mouse models of disease, allowing for studying human mitochondrial function *in vivo*.

Mitochondria are the powerhouses of the cell. Would mitochondria also be the engines of growth that confer developmental competence in stem cells? Our view about how stem cells are regulated has been largely nucleus-centric. Little is known about the role of mitochondria in the regulation of stem cell behavior. With the iPS cell technology, we can seek to determine the variability in human mitochondrial structure and function in different iPS cell lines by reprogramming human somatic cells from healthy individuals at different ages or people with inherited mitochondrial diseases, to gain an appreciation for the effects of alteration in mitochondrial structure and function on cell behavior, and also as a way to help to select appropriate cells for future therapeutic applications. It would be interesting to compare side-by-side iPS cells with hES cells. We can force hESC/iPSCs with normal or altered mitochondria to differentiate into various cell lineages to evaluate how the state of mitochondrial structure and function will dictate the ability for hESC/iPSCs to provide multiple, distinct replacement lineages for use. We can determine whether alterations in mitochondrial fusion and fission dynamics critically regulate hESC/iPSC pluripotency and fate choice. In addition, we can seek to determine whether we can use hESC/iPSCs with functional mitochondria to repair cells with nonfunctional mitochondria. Taking advantage of a recent breakthrough technology in reprogramming human somatic cells back to pluripotent stem cells, together with tools in mitochondrial imaging and

analysis of dynamics and function, we should be able to shed light on how mitochondria regulate stem cell behavior in development and disease. This line of research is an unexplored area of investigation and represents a paradigm shift in mitochondrial research. The knowledge to be obtained from this line of research would likely be critical for enhancing our ability to manipulate hESC/iPSCs for designing efficient cell-based therapies, and to understand human mitochondrial function in development and disease. Although largely exploratory in nature, the unprecedented integration of new perspectives and approaches to study human mitochondrial function by this reverse engineering approach via iPSC cell generation would transcend this area of research, and would likely lead to important results that have tremendous research and medical implications.

### **Proving the “king of all cells” property (pluripotency): iPSC cells yield live mice**

*Bona fide* stem cells are considered as “kings of all cells”. A trio of recent papers<sup>[32-34]</sup> has finally shown that the feat of creating live mice from iPSC cell derived from skin cells is possible. Generation of live mice entirely from iPSC cells is the ultimate test of their developmental pluripotency. Stem cells are unique in their ability to develop into different cell types in the body. This property of stem cells has great potential value in the discovery of new drugs and therapies for many diseases. The creation of stem cells lines with specific genetic makeup that predisposes, or leads to disease, is an essential tool for the development of therapies for human diseases. It is also important to study stem cells representing a diversity of genetic backgrounds, since different patients respond differently to a given therapy. A goal in translational stem cell medicine is to harness the power of iPSC cells to reproducibly differentiate into essential cell types, e.g. heart, blood, neural cells, for drug discovery and development and eventually therapeutic use.

### **“Oh Rats! The Story of Rats and People”**

A comic book with such an intriguing title depicts vividly the similarities in adverse social behavior between rats and humans. Interestingly enough, the story is that indeed there is also a truth about the similarities in physiology between rats and humans. Numerous studies have shown that humans are more similar to rats than mice in many aspects of physiology and metabolism<sup>[36,37]</sup>. However, because of the availability of transgenic mouse models, mice but not rats have been used as model systems to study human physiology and disease. Only

recently, rat ES cells have been successfully derived<sup>[36,37]</sup>. However, these ES cells might not be the only route to genetically engineered rats. Rat iPSC cells have been generated by reprogramming adult rat cells, including from liver, skin, and bone marrow cells<sup>[38]</sup>. These iPSC cells can be used to make transgenic rats, given the recent success of creating live mice from iPSC cells, thus opening the door to additional model systems that are more feasible in rats than in mice.

### **Unleashing the therapeutic potential of iPSC cells: Science becomes medicine**

The amazing breakthrough in stem cell biology is that human non-embryonic cells can be reprogrammed to behave like embryonic stem cells. These induced pluripotent stem cells have the ability to divide without differentiating for a prolonged period, to give rise to derivatives of all three embryonic germ layers, and to yield live mice. The iPSC cells provide a unique opportunity to study how somatic cells de-differentiate to an embryonic stem cell-like state, and therefore also to understand the molecular basis of cell differentiation from the pluripotent state. The iPSC cells share many features with human ES cells, but may not be identical<sup>[39]</sup>. Furthermore, induction of iPSC cells still has a very low efficiency and a number of safety concerns. The intermediate molecular and biochemical steps in the reprogramming process of iPSC cell generation are largely unknown. Thus, while iPSC cells have enormous potential to substitute for ES cells on multiple fronts and to generate genetically diverse and patient-specific pluripotent stem cell populations, more research will be needed to realize this potential.

The iPSC cell technology enables to represent the diversity of human biology in an *in vitro* test by making personalized pluripotent stem cells from any individual with different risk factors and predispositions to disease. As the progress in stem cell biology promises to bring about new revolutions to drug discovery, it can be expected that the current drug discovery efforts will ultimately lead to exciting, unanticipated results and bear fruits in the future. Despite many potential pitfalls, the iPSC cell technology, although still nascent, represents a remarkable step forward toward development of models for human disorders, drug discovery and development, and therapeutic applications. Recent studies have shown the initial success of 20 different disease-specific iPSC cell lines<sup>[40]</sup> and patient-derived iPSC cells for amyotrophic lateral sclerosis<sup>[41]</sup>, spinal muscular atrophy<sup>[42]</sup>, Parkinson's disease<sup>[12]</sup>, Fanconi anaemia<sup>[43]</sup>, and familial dysautonomia<sup>[44]</sup> as wonderful proof-of-concept for the utility of

the iPS cell technology. What's here and now is a true stem cell revolution. The time has never been better to unleash the therapeutic potential of stem cells in drug discovery and regenerative medicine.

## References

1. Takahashi K and Yamanaka S. *Cell*. 2006, 126(4):663-76.
2. Takahashi K, et al., *Cell*. 2007, 131(5):861-72.
3. Yu J, et al., *Science*. 2007, 318(5858):1917-20.
4. Park IH, et al., *Nature* 2008, 451(7175):141-6.
5. Nakagawa M, et al., *Nat Biotechnol.*, 2008, 26(1):101-6.
6. Hanna J, et al., *Science* 2007, 318(5858):1920-3.
7. Carey BW, et al., *Proc Natl Acad Sci U S A*, 2009 106(1):157-62.
8. Stadtfeld M, et al., *Science* 2008, 322(5903):945-9.
9. Okita K, et al., *Science* 2008, 322(5903):949-53.
10. Woltjen K, et al., *Nature* 2009, 458(7239):766-70.
11. Kaji K, et al., *Nature* 2009, 458(7239):771-5.
12. Soldner F, et al., *Cell* 2009, 136(5):964-77.
13. Yu J, et al., *Science* 2009, 324(5928):797-801.
14. Zhou H, et al., *Cell Stem Cell* 2009, 4(5):381-4.
15. Kim D, et al., *Cell Stem Cell* 2009, 4(6):472-6.
16. Xu Y, et al., *Nature* 2008, 453(7193):338-44.
17. Rubin LL. *Cell* 2008, 132(4):549-52.
18. Sul JY, et al., *Proc Natl Acad Sci U S A*. 2009, 5;106(18):7624-9.
19. Do JT and Schöler HR. *Trends Pharmacol Sci*. 2009, 30(6):296-302.
20. Mikkelsen TS, et al., *Nature* 2008, 454(7200):49-55. Erratum in: *Nature* 2008, 454(7205):794.
21. Ahel D, et al., *Science* 2009, PubMed PMID: 19661379.
22. Gottschalk AJ, et al., *Proc Natl Acad Sci U S A*. 2009, PubMed PMID: 19666485;
23. Gaspar-Maia A, et al., *Nature* 2009, 460(7257):863-8. Epub 2009 Jul 8. PubMed PMID: 19587682.
24. Timinszky G, et al., *Nat Struct Mol Biol*. 2009, PubMed PMID: 19680243.
25. Hong H, et al., *Nature* 2009, PubMed PMID: 19668191.
26. Utikal J, et al., *Nature* 2009, PubMed PMID: 19668190.
27. Marión RM, et al., *Nature* 2009, PubMed PMID: 19668189.
28. Li H, et al., *Nature* 2009, PubMed PMID: 19668188.
29. Kawamura T, et al., *Nature* 2009, PubMed PMID: 19668186.
30. Banito A, et al., *Genes Dev*. 2009, PubMed PMID: 19696146.
31. Gao F, et al., *J Biol Chem*. 2009, 284(33):22263-73.
32. Zhao XY, et al., *Nature*. 2009, PubMed PMID: 19672241.
33. Kang L, et al., *Cell Stem Cell* 2009, 5(2):135-8.
34. Boland MJ, et al., *Nature* 2009, PubMed PMID: 19672243.
35. Ramalho-Santos M. *Cell* 2009, 138: 617-618.
36. Li P, et al., *Cell* 2008, 135(7):1299-310.
37. Buehr M, et al., *Cell* 2008, 135(7):1287-98.
38. Liao J, et al., *Cell Stem Cell*. 2009, 4(1):11-5.
39. Chin MH, et al., *Cell Stem Cell* 2009, 5(1):111-23.
40. Park IH, et al., *Cell* 2008, 134(5):877-86.
41. Dimos et al., *Science* 2008, 321:1218-1221.
42. Ebert et al., *Nature* 2008, 457:277-280.
43. Raya A, et al., *Nature* 2009, 460(7251):53-9.
44. Lee G, et al., *Nature* 2009, PubMed PMID: 19693009.