Exploiting pluripotency for therapeutic gain

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Human embryonic stem cells (hESCs) have been recognized as the “gold standard” for research on pluripotency and differentiation, and hold great promise for advancing our knowledge of human development, biology, disease and therapy. However, 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 pluripotent cells by using defined “reprogramming factors”. While these reprogrammed cells have similar developmental potential to authentic hESCs, they are not derived from human embryos, and are thus termed “Induced pluripotent stem cells (iPSCs)”. The iPSC technology would prove useful for generation of individual cell lines from many different patients to study the nature and complexity of disease. Moreover, problems of immune rejection for future therapeutic applications would be greatly relieved by being able to generate reprogrammed cells from individual patients. Although iPSC generation is still slow, inefficient, fraught with pitfalls, and unsafe for human use, recent research has yielded exciting insights into the understanding of the technology, logic, safety, and utility of iPSCs, and has led to the use of these unique cells for disease modeling, drug discovery and regenerative medicine, paving paths to new therapeutics.

KEY WORDS: Stem cell - Human embryonic stem cells - Induced pluripotent stem cells - Regenerative medicine.

Acknowledgements: The author would like to thank David Pleasure, MD, for his critical comments. The work was jointly supported by grants from National Institutes of Health (RO1 NS09083 and RO1 ES015988), National Multiple Sclerosis Society, Feldstein Medical Foundation, and Shriners Hospitals for Children. The author declares no competing interests related to this article.

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Human embryonic stem cells (hESCs):
Still the “gold standard”

Although animal models have been invaluable for disease research and drug discovery applications, 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. Agents predicted as “safe” from animal studies might elicit unbearable adverse events in humans. Thus, limitations of animal models to elucidate human response should not be overlooked, and new models with greater predictive ability and mechanistic insight into human development and function are greatly needed. 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.

Adult (endogenous) stem cells generally have a limited repertoire of developmental choices and are often called multipotent, while stem cells from early embryonic stages have broader developmental potential to differentiate into all of the cell types in the body and are thus defined as pluripotent. The difficulties in obtaining a pure population of adult stem cells and in expanding these cells to sufficient quantities while maintain-
ing the progenitor state remain significant obstacles for their use as cell sources for human therapy or drug discovery/toxicity testing platforms. hESCs are capable of self-renewing and differentiating into all cell types in the human body, and are potentially an unlimited cell sources for these applications. Furthermore, hESCs are still the “gold standard” for use. Faithful recapitulation of human development and disease has been demonstrated via differentiation of hESCs. The value of hESCs to predict if and how drugs work and cause toxicity or disrupt development has been validated by an increasing body of evidence. 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. In addition, 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 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.

**Induced pluripotent stem cells (iPSCs): A catalyst for new medicines**

The use of iPSCs remains controversial and poses ethical considerations since current isolation techniques result in the destruction of the embryos from which the cells were obtained. Another 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.

A recent breakthrough in stem cell biology is the success of converting differentiated human somatic cells into ESC-like cells, termed induced pluripotent stem cells (iPSCs), by using defined “reprogramming factors” (Takahashi et al., 2006, 2007; Yu et al., 2007; Park et al., 2008a). The success of cell reprogramming has opened a new field of biology and holds out hope of life-saving medical advances. The iPSC technology is becoming a major tool for modeling disease and drug discovery. Moreover, if cell reprogramming becomes more efficient and safer, patients might someday be treated with healthy versions of their own cells, avoiding immune rejection in therapy. The remarkable developmental potential of iPSCs promises to introduce a new era of science and medicine, and has given rise to the compelling dream of being able to restore function after disease or injury by replacing damaged cells with healthy new cells. These unusual cells might someday provide the cellular components for the even more ambitious task of reconstructing entire organs, and offer a powerful tool for understanding a variety of diseases, so that targets for new therapeutics can be defined.

**The “LUCK” of iPSCs: Logic, Utility, Concern, and “King of all cells” property (i.e., pluripotency)**

The Yamanaka group first described the identification of the combination of four transcription factors whose retroviral overexpression enabled the induction of a pluripotent state in somatic cells such as skin fibroblasts; the remarkable finding was that simultaneous overexpression of Oct4, Sox2, Klf4 and c-Myc led to generation of iPSCs that were similar to ESCs (Takahashi et al., 2006, 2007). The Thomson group also reported success in in vitro reprogramming of human fibroblasts to iPSCs with lentiviral overexpression of another combination of reprogramming factors (Oct4, Sox2, Nanog, and Lin28) (Yu et al., 2007).

The iPSC is truly an amazing story. Although Shinya Yamanaka’s invention of iPSCs in 2006 (Takahashi et al., 2006) was far from serendipity, I suspect that even the man, who started it all to create this field himself, would admit sometimes a bit of “luck” might be helpful in the pursuit of groundbreaking science. In this context, here I discuss the “LUCK” (Logic, Utility, Concern, and “King of all cells” property) of iPSCs.

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

The iPSC technology provides a unique opportunity to understand the molecular basis of somatic cells
de-differentiate to a pluripotent state. The intermediate molecular and biochemical steps in the reprogramming process are largely unknown. Understanding the sequence of changes in cell reprogramming would provide dynamic trajectories of molecular states between cell states that would aid in deciphering the molecular logic underlying cell reprogramming. Such an understanding of the molecular map has the potential to crack the "black box" of cell reprogramming and transform regenerative medicine with far-reaching applications, where various cell sources can be turned into model systems for tissue-specific regenerative processes and therapeutics.

Various cutting-edge genomic and proteomic profiling techniques have been used to monitor the earliest changes that take place and identify the critical factors that lead to reprogramming of the human transcriptome. For example, Mikkelsen et al. (2008) studied how the regulatory network is used by iPSCs, and interrogated this question on the genome scale with ChIP-chip, a technique that combines chromatin immunoprecipitation ("ChIP") with microarray technology ("chip") to investigate interactions between proteins and DNA and to identify binding sites for DNA-binding proteins, and with ChIP-Seq, which uses high-throughput sequencing to detect and quantify immunoprecipitated DNA fragments bound by transcription factors. With such whole-genome data sets, including ChIP-chip and ChIP-Seq of multiple transcription factors, maps of epigenetic states, expression profiling of genetically manipulated cells, protein-protein interactions, and complementary studies in mouse and human species, it should be possible to construct comprehensive wiring diagrams for somatic cell reprogramming. Importantly, a more thorough understanding of the regulatory network should also suggest rational use of small-molecule agents for cell reprogramming, such as chemical inhibitors of histone modifications and DNA (de)methylation, which might alter genome-wide patterns of epigenetic modifications, thereby preventing potentially unintended consequences (Xu et al., 2008). In addition, candidate molecules identified by functional genomic and proteomic analyses could be validated by loss-of-function and gain-of-function studies, and then tested for their ability to enhance reprogramming efficiency in various methods of inducing pluripotency, such as nuclear transfer or reprogramming induced by "reprogramming factor" genes.

To realize the potential of medical applications of iPSCs, it is of utmost importance to delineate the mechanism of cell reprogramming and to use this information to develop efficient and safe methods for iPSC generation. Recently, Bhutani et al. (2010) developed a cell fusion strategy in which reprogramming was initiated rapidly and efficiently, representing a fast track of reprogramming towards pluripotency. The researchers fused mouse embryonic stem cells with human skin cells to create hybrids called heterokaryons, leading to quick and efficient reprogramming. Key advantages of this fusion-based approach included rapid access to the earliest of reprogramming events and a more efficient and synchronous environment by which specific pluripotency mechanisms could be uncovered.

The concerns: Addressing the risk and pitfalls of current iPSC technologies

Presently, the commonly used methods for iPSC generation involve the procedure of introducing viruses and random gene insertions into target cells, which is dangerous and can lead to tumors and other abnormalities in the cells, and also presents challenges that are likely to complicate or preclude the full range of potential applications of this approach. As a result, iPSCs created to date are not suitable for use in the clinic. Novel methods are greatly needed to create iPSCs without the use of viruses and free of unsafe genetic manipulations. Success in such approaches will provide useful and safe methods to generate reprogrammed stem cells, and will speed the use of these cells in a wide variety of drug discovery and clinical applications.

Genome integrity might be a fundamental issue for reprogrammed cells. Although most iPSCs appear to have normal karyotypes, the levels of genomic mutations in iPSCs remain to be examined by detailed genomic analyses, such as whole genome sequencing. It is possible that genetic mutations disrupting tumor suppressors, such as p53, may accumulate in iPSCs, and thus iPSCs may be intrinsically genetic unstable. Whole genome sequencing will help determine the tumorigenicity of iPSCs. Such analyses would pick up any variation between the patient's genome and an established iPSC line. Arguably, stability should be the most important criterion of quality control for iPSC lines generated from patients, as the patient and the resulting cells have the same genome. However,
even when human iPSC lines are generated without viruses or through nuclear transfer, the act of reprogramming and culturing might still introduce selective pressures that favor certain genetic alterations over others.

Although the "triggers" of cell reprogramming have been identified, the efficiency is extremely low, suggesting the existence of "barriers" that impede cell reprogramming. Recent studies have identified that p53, often dubbed the "guardian of the genome," represses reprogramming, and inactivation/inhibition of p53 breaks the barrier and increases the efficiency of iPSC generation (Deng and Xu, 2009; Krizhanovsky and Lowe, 2009). These findings suggest that reprogramming somatic cells into iPSCs could be at the expense of genome stability, suggesting that mechanisms controlling tumor progression overlap those controlling stem cell pluripotency. Thus, cancer might develop when destroying such iPSC barriers as p53 that is also critical for maintaining genome integrity and chromatin plasticity. A challenge is to strike a delicate balance between efficiency and untoward risk.

The utility: Using the iPSC technology for reverse engineering human disease and as an exploratory tool

The ability to de-differentiate patient-specific adult cells back to stem cells and then to re-differentiate them into specific lineages would allow creation of 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. Recent studies have demonstrated the initial success of patient-derived iPSCs as wonderful proof-of-concept for the utility of the iPSC technology (Hamal et al., 2007; Park et al., 2008b; Dimos et al., 2008; Ebert et al., 2008; Ray et al., 2009; Lee et al., 2009). This technology would prove useful for the generation of individualized stem cells from many different patients to study the nature and complexity of disease, and would undoubtedly catalyze progress in understanding pathogenesis of and developing treatments for human disease. For years, researchers have grown human somatic 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. Disease-specific stem cell cultures would harbor the disease genome of the donor. 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. Disease-specific stem cells can enable us 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 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.

The iPSC technology is also an enabling, exploratory tool; it is a vehicle for many tantalizing possibilities of technological developments, and represents a platform for a wide variety of lines of basic and applied biology investigations. For example, the iPSC method can be an innovative tool for human mitochondrial research, since we can generate iPSCs with normal or abnormal mitochondria from healthy people at different ages and patients with different mitochondrial genetic diseases, and then force the iPSCs to differentiate into various cell lineages to evaluate how the state of mitochondrial structure and function will dictate the ability of the iPSCs to provide distinct lineages. In addition, combining human iPSCs 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 iPSCs into mice would thus create humanized mouse models of disease, allowing for studying human mitochondrial function in vivo. Indeed, our view about how stem cells are regulated has
been largely nucleus-centric, and little is known about the role of mitochondria in the regulation of stem cell behavior. As mitochondria are the powerhouse of the cell, would mitochondria also be the engines of growth that confer developmental competence in stem cells? As mitochondrial fusion and fission dynamics can drive cell cycle (Mitra et al., 2009), alterations in this machinery, e.g., those occur in normal versus mitochondrially diseased iPSCs, might critically regulate pluripotency and fate choice in iPSCs. Taking advantage of the iPSC technology in reprogramming human somatic cells from patients with mitochondrial disease back to pluripotent stem cells, together with tools in mitochondrial imaging and analysis of dynamics and function, we should be able to shed fresh light on how mitochondria regulate stem cell behavior in human development and disease.

In addition, iPSCs generated from various animal species can be exploited to develop appropriate model systems for studying human physiology and disease. iPSCs have been generated from rats and several other species including large animals such as pigs and monkeys. “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, there is indeed 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 (Li et al., 2008; Buehr et al., 2008). However, because of the availability of transgenic mouse models, mice but not rats have been widely used as model systems to study human physiology and disease. Only recently, rat ESCs have been successfully derived (Li et al., 2008; Buehr et al., 2008). However, these ESCs might not be the only route to genetically engineered rats. Rat iPSCs have been generated by reprogramming adult rat cells, including from liver, skin, and bone marrow cells (Liao et al., 2008). These iPSCs can be used to make transgenic rats that could become the “next top model”, thus opening the door to additional model systems that are more feasible in rats than in mice.

The “king of all cells”: iPSCs yield live mice and prove ultimate pluripotency

Bona fide stem cells are considered as the “king of all cells”. A trio of recent papers (Zhao et al., 2009; Kang et al., 2009; Boland et al., 2009) has finally shown that the feat of creating live mice from iPSCs derived from skin cells is possible. Generation of live mice entirely from iPSCs is the ultimate test of their developmental pluripotency. These fully pluripotent iPSCs and iPSC-derived mice would allow for investigation into the functional stability of iPSC-derived tissues and to probe the genomic stability of differentiated cells. Knowledge acquired from this research would help generate improved iPSC lines. A goal in translational stem cell medicine is to harness the power of available technologies to identify and reproducibly differentiate reliable iPSC lines into essential cell types, e.g., heart, blood, pancreatic and neural cells, for drug discovery and development and eventually therapeutic use.

Unleashing the therapeutic potential of iPSCs: Science becomes medicine

While iPSCs have enormous potential to substitute for ESCs on multiple fronts and to generate genetically diverse and patient-specific pluripotent stem cell populations, more research will be needed to realize this potential. iPSCs share many features with ESCs, but may not be identical (Chin et al., 2009). Furthermore, induction of iPSCs still has a very low efficiency and a number of safety concerns. The derivation of iPSCs that requires genetic modification using DNA transduction to introduce reprogramming genes will most likely cause permanent genetic modifications that can perturb cell fate in unpredictable ways. Given the fear of the oncogenic risk associated with these iPSCs, intense efforts were directed towards sweating technological improvements for safer iPSCs (Stadtfeld et al., 2008; Okita et al., 2008; Woltjen et al., 2009; Kaji et al., 2009; Soldner et al., 2009; Yu et al., 2009). Protein-based reprogramming methods were developed and implemented (Zhong et al., 2009; Kim et al., 2009) as a way of programming cell fate without genetic modifications. However, protein-based methods are difficult to scale up and with extremely low efficiency, and might be incompatible with the need to generate various individual cell lines from many different patients to study the nature and complexity of disease and for individualized cell-based therapy. Purely chemical-based reprogramming methods have not been accomplished to date, but there have been reports of a number of small molecules capable of replacing genes in the reprogramming cocktail or...
enhancing iPSC generation. One interesting and promising finding was the ability of Vitamin C to boost reprogramming efficiency (Esteban et al., 2010).

A stem cell-based therapeutic approach is very attractive for many devastating human disorders, often representing the best hope for medical breakthroughs that will reduce human suffering from these diseases. While hESCs hold great potential in regenerative medicine and drug discovery, the development of iPSCs could provide an ideal cell source for transplantation by avoiding graft rejection in the patient, and 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 research is to resolve the major bottlenecks remaining in human iPSCs to make it feasible for drug discovery and safe for human use. Despite many potential pitfalls, the iPS technology, although still nascent, represents a remarkable step forward toward development of models for human disorders, drug discovery and development, and therapeutic applications. What’s here and now is a true stem cell revolution, and the time has never been better!

References


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