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REVIEW

Induced Pluripotency for Translational Research

Menghua Wu, Guilai Chen, Baoyang Hu *

State Key Laboratory of Reproductive Biology and Key Laboratory of Translational Stem Cell Research, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China

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Abstract The advent of induced pluripotent stem cells (iPSCs) has revolutionized the concept of cellular reprogramming and potentially will solve the immunological compatibility issues that have so far hindered the application of human pluripotent stem cells in regenerative medicine. Recent findings showed that pluripotency is defined by a state of balanced lineage potency, which can be artificially instated through various procedures, including the conventional Yamanaka strategy. As a type of pluripotent stem cell, iPSCs are subject to the usual concerns over purity of differentiated derivatives and risks of tumor formation when used for cell-based therapy, though they provide certain advantages in translational research, especially in the areas of personalized medicine, disease modeling and drug screening. iPSC-based technology, human embryonic stem cells (hESCs) and direct lineage conversion each will play distinct roles in specific aspects of translational medicine, and continue yielding surprises for scientists and the public.

Introduction

The reversion of differentiated cells to a state of pluripotency is a fascinating idea that has long been explored in cell biology, yet reversion to pluripotency simply through the over-expression of a set of pluripotency-associated factors in somatic cells appeared to be impossible before Yamanaka and his colleagues successfully reprogrammed mouse fibroblasts to pluripotent stem cells, the so-called induced pluripotent stem cells (iPSCs). These cells exhibit the morphology and growth properties of embryonic stem cells (ESCs) and express endogenous ESC markers, such as Oct4 and Nanog [1]. This landmark breakthrough quickly evoked the enthusiasm of both scientists and the public toward stem cells because of their far-reaching scientific value and numerous potential applications. In this review, we summarize recent advances in the field of reprogramming and iPSCs, in particular the new conceptual framework of cell fate determination and its potential applications in translational research.

From somatic cell nuclear transfer to iPSCs

Induced pluripotency has been studied for a very long time. In the 1950s, Briggs and King established the technique of somatic cell nuclear transfer (SCNT), or "cloning", by transplanting isolated nuclei into enucleated oocytes [2,3]. Using this system, they successfully cloned tadpoles from cell nuclei of late-stage embryos and tadpoles. In the early 1960s, John Gurdon transplanted nuclei of adult frog intestinal cells into

* Corresponding author.
E-mail: byhu@ioz.ac.cn (Hu B).

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unfertilized eggs and generated tadpoles [4,5]. Despite the pioneering success of SCNT in the amphibian, it was not until the late 1990s that Ian Wilmut and colleagues cloned the first mammal, Dolly the sheep [6]. This work demonstrated that differentiated somatic cells indeed retain the genetic information that is necessary for the generation of a multicellular organism, and during development, reversible epigenetic rather than irreversible genetic changes are imposed on the genome, most possibly by factors in the oocytes.

Another remarkable breakthrough in life science accompanying SCNT was the derivation of ESCs from the inner cell mass (ICM) of mouse and human blastocysts [7–9]. In an optimal culture condition that enables the long-term maintenance of pluripotency, ESCs, the *in vitro* counterparts of ICM cells, can be propagated indefinitely [10]. This has allowed the in-depth dissection of pluripotency circuitry and identification of the master pluripotency genes Oct4, Nanog and Sox2, which have been employed to generate iPSCs.

Just as ESCs, the properties of differentiated cell lineages are determined by "master" genes necessary for establishing and maintaining cellular identity. Products of these master genes drive the expression of cell type-specific genes while suppressing lineage-unrelated genes. Ectopic expression of these master genes can induce a cell fate change. In *Drosophila*, ectopic expression of the transcription factor Antennapedia in the head region results in the formation of legs instead of antennae. Overexpression of skeletal muscle determinant gene MyoD in the mouse fibroblasts results in the formation of myocytes [11,12]. Upon overexpression of the myeloid transcription factor C/EBPa, primary B and T cells efficiently convert to functional macrophages in mice [13,14]. These findings suggest that transcription factors play key roles in cell fate determination, and that ectopic expression of such factors can switch the cell fates of differentiated cells.

Yamanaka and Takahashi devised a screening system that could activate a dormant drug resistance allele that was integrated into the ESC-specific *Fbxo15* locus and selected from a pool of 24 candidate pluripotency-associated genes. They found that only four of the factors, Oct3/4 (also known as Pou5f1), Sox2, Klf4 and c-Myc, were needed to generate ESC-like colonies from fibroblasts of both embryonic and adult mice. They termed these reprogrammed cells 'induced pluripotent stem (iPS) cells' [1]. However, it was later demonstrated by Yamanaka's group and other investigators that these iPSCs were not fully reprogrammed, since iPSCs selected through this approach failed to produce adult chimaeras. It was soon recognized that *Fbxo15* was not an ideal selection gene, and thus later, with the use of *Nanog* or *Pou5f1* instead of *Fbxo15* for selection, germline-competent iPSCs very similar to ESCs were generated in multiple labs [15–17]. A few years after iPSCs were initially developed, the last skeptics were finally convinced by a stringent verification of iPSC pluripotency: individual iPSCs were able to generate viable mice in a tetraploid compensation assay [18]. At the same time, human iPSCs (hiPSCs) were induced using the same or a similar set of transcription factors, and subsequently were widely used for disease modeling and drug screening [19–21]. The stem cell research field was then boosted by the emergence of hot topics such as probing the mechanisms of reprogramming, increasing reprogramming efficiency and improving therapeutic safety [22–25].

All roads lead to cell fate change

During mammalian development, cells gradually lose potential and become progressively differentiated to fulfill the specialized functions of somatic tissues. The traditional Waddington's concept of the 'epigenetic landscape' described a progressively restricted and educative hierarchical model of cell differentiation potential during normal development. According to this model, the pluripotent state resides 'above' the differentiated somatic states, and lineage differentiation and commitment are unidirectional and irreversible. Pluripotency-associated factors and lineage specifiers have divergent roles in maintaining identities of pluripotent or differentiated states [26]. SCNT and transcription factor-based reprogramming experiments demonstrated that a terminally-differentiated somatic cell fate can be reversed, yielding a pluripotent state. During reprogramming or direct lineage conversion, the cells need to overcome the epigenetic hierarchy or the barriers between the lineages. It was not until recently that Shu et al. revealed that balanced overexpression of transcription factors that control ectoderm and mesendoderm lineage specification can also reprogram the mouse fibroblasts into iPSCs. They proposed a "seesaw" model to explain their findings: when all specification forces are well balanced at an appropriate level, the reprogrammed cells are allowed to assume a pluripotent state [27]. This is in agreement with other findings that preventing lineage specification is sufficient for pluripotency induction. Although the precise mechanism by which lineage specifiers coordinate the induction of pluripotency is still under investigation, the insights that have already emerged in this regard have enhanced our understanding of the true nature of pluripotency.

Based on a careful analysis of the literature on direct reprogramming, Ladewig et al. proposed an epigenetic disc model of cell fate change, which seems more adaptable to somatic cell fate conversion, including iPSC induction [28]. In this model, the pluripotent state locates in the central area of a flat disk, represents just one of many possible states of a cell, and is metastable, requiring certain conditions for long term maintenance. In the case of a cell fate change, a cell has multiple choices in terms of its destination, and can proceed through a shortcut to one cell fate or alternative routes to reach a different cell fate. The non-hierarchical 'epigenetic disc' model extends our understanding of cell fate change and will facilitate the development of optimized approaches for cell differentiation, reprogramming and trans-differentiation. Although pluripotency induction seems feasible according to this model, it reminds us that a wide variation in pluripotency might exist among different iPS cell lines, which needs to be carefully considered when they are used for research and discovery.

Since reprogramming factors such as Oct4, Sox2, c-Myc and Klf4 regulate specific signaling pathways, it is conceivable that different combinations of small molecules can be used to reprogram somatic cells. Although the complete chemical reprogramming approach remains to be further explored for reprogramming of human somatic cells, chemically induced pluripotent stem cells (CiPSCs) have already been generated from mouse somatic cells, using a combination of seven small molecule compounds [29]. These findings increase our understanding about the establishment of cell identities and open

up the possibility of generating functionally desirable cell types for regenerative medicine, using specific chemicals or drugs, instead of genetic manipulation and difficult-to-manufacture biologics. As the reprogramming strategies are improved, we will be equipped to tackle challenges that have hampered the use of iPSCs in clinical and translational medicine.

hiPSCs can and can't

Similar to hESCs, iPSCs have the ability to proliferate indefinitely and differentiate into any cell types of the body. These features make iPSCs an attractive complement to hESCs in many aspects of research and translation, in particular disease modeling, and a potential source of cells for personalized regenerative medicine. However, as a novel type of cell still at an early stage of scientific study, plenty of issues exist that limit the application of hiPSCs. The techniques for reprogramming are far from optimized; mutations during reprogramming may cause abnormalities in the iPSC lines; and the differentiation potential of iPSC lines may vary. At the top of the task list for promoting the application of iPSCs is refining the reprogramming technique, for example, using small molecules to generate genomic non-integrative iPSCs.

Ideal model for studying human development

hiPSCs, like ESCs, are invaluable tools for studying human development. Because their *in vitro* differentiation faithfully recapitulate what occurs in *in vivo* development, and iPSC lines usually retain the same genetic information with their donors. hiPSCs provide certain advantages in the study of neural development, especially early neural system development. The development and optimization of protocols for directed differentiation have made it easy for investigators to differentiate iPSCs into many subtypes of neurons with the course of differentiation mimicking the endogenous human neural development process. The hiPSCs can be converted to neuroepithelial cells (NE cells), and these hiPSC-derived NE cells can then "pattern" efficiently to region-specific neural progenitors along the anterior-posterior axis, which can further differentiate into functional neurons including forebrain glutamatergic neurons, midbrain dopaminergic neurons and spinal motor neurons [30–32]. Most of these protocols have been developed based on our knowledge of developmentally-relevant signals identified in animal models. hiPSCs and animal models complement each other, thus promoting the understanding of the mechanisms of developmental processes. As for disease-based iPSCs, especially those developmental disease-based iPSCs, they are ideal tools for studying the early events relevant to the development of the specific diseases. By studying molecular defects or mutations that are readily observable in iPSC-derived cells, we are able to investigate the important roles of the affected molecules and identify how particular molecular events affect normal development. For example, using iPSCs generated from the fibroblasts of a patient with Rett syndrome (RTT) as models, investigators identified an unexplored critical window at the early stage of neural development, during which subtle alterations in the nervous system, found to be caused by MeCP2 mutations, play important roles in the initiation of RTT [33].

Feasible system for disease modeling and drug screening

Because of the limitations of animal models, human specific aspects of diseases are hard to clarify. Mechanistic findings and therapeutic approaches for animal models usually failed to be translated into a human context. Patient-specific iPSCs provide a unique platform to study human genetic diseases *in vitro*, particularly for inherited developmental disorders. Through differentiation of iPSCs along specific lineages, some of the phenotypes of mono-gene diseases have been recapitulated in a dish. In addition, for some of the more complex polygenic disorders, patient-specific iPSCs also proved to be useful models of disease progression. As a model system, iPSCs and *in vitro* differentiation can be used to explore pathogenesis, develop early diagnostic tools and discover potential treatment approaches. A variety of patient-specific hiPSCs from Parkinson's disease (PD) [34], Alzheimer's disease (AD) [35–37], Huntington's disease (HD) [38] and schizophrenia [39] patients have been obtained and have all been shown competent to model the disease progression *in vitro*. In most cases, iPSCs were differentiated into disease-relevant subtypes of cells exhibiting certain disease features. Using hiPSCs from familial and sporadic AD patients, researchers have successfully established the AD model and revealed stress phenotypes associated with intracellular Aâ in neurons/astrocytes and differential drug responsiveness [40]. Similarly, from somatic cells of a late stage pancreatic ductal adenocarcinoma (PDAC) patient, iPSCs were generated and re-differentiated into pancreatic tissue [41]. These disease-specific iPSC-derived pancreatic cells mimic the progression of early to mid-stage pancreatic cancer, releasing protein which later was identified as a biomarker of early-stage cancer progression. Identification of biomarkers for such cancers will eventually facilitate the early detection and successful treatment of these diseases, and thus potentially reduce associated mortality.

Usually, conventional drug discovery is costly and time-consuming. In addition, a large proportion of candidate drugs that have passed animal tests fail testing in the following stages, largely because of efficacy and safety issues when used in humans. Thus most animal model-based pre-clinical studies lead to uncertain results in clinical trials, which is a huge problem for the pharmaceutics industry. To help overcome this issue, iPSCs and the differentiated derivatives that recapitulate disease phenotypes can be used for stem cell-based drug screening. Compared to other systems such as animal models, hiPSCs offer unique advantages. They directly provide information on how drugs affect human cells; iPSC-based screening is much easier to operate on a large scale; disease-specific iPSCs have higher sensitivity and accuracy; and iPSC-based screening is cost effective. In an attempt to identify effective drugs for the treatment of PD, investigators found that only 16 out of 44 compounds shown effective in animal models were able to protect human stem cell derived-dopaminergic neurons from rotenone-induced cell death [42], a result indicating the need to use disease-relevant human neurons for drug screening. In a high-throughput drug screen, 8 out of 6912 small molecule compounds tested on neural crest precursors derived from familial dysautonomia (FD) iPSCs proved able to rescue phenotypes of the disease to a level similar to that observed in cells with wt-IKBKAP, the gene that is responsible for FD. Among these compounds, SKF-86466 could induce IKBKAP

transcription through modulation of the levels of intracellular cAMP and PKA-dependent CREB phosphorylation. SKF-86466 was also able to rescue the expression of IKAP protein and disease-specific loss of autonomic neuronal marker expression [43]. In another study, researchers employed the stem cell-based drug screening techniques and found that the survival of motor neurons was greatly improved upon treatment with a compound called kenpaullone, which is much cheaper and more effective than olesoxime and dexpramipexole, two drugs that are currently used to treat amyotrophic lateral sclerosis (ALS) patients [44].

Uncertain cell-based therapy

Another exciting aspect of iPSCs is the possibility that custom-tailored pluripotent cells can be generated for autologous cell transplantation, as has been indicated by a compelling study showing that sickle cell anemia model mice can be rescued by transplantation of hematopoietic progenitors differentiated from autologous iPSCs [45]. In the central nervous system, transplantation of hiPSC-derived oligodendrocyte progenitor cells (OPCs) into the neonatal brains of myelin-deficient shiverer mice resulted in a robust myelination of the hypomyelinated shiverer brain and substantially increased host survival with no evidence of either tumorigenesis or heterotopic non-glial differentiation [46]. These studies indicate that transplantation of iPSC derivatives for customized therapeutic regeneration is feasible.

Although iPSCs have possible applications in personalized clinical intervention, there are still challenges in using iPSCs for translational applications. One problem in using iPSC-derived cells for transplantation is that residual undifferentiated cells increase the risk of teratoma formation. Another obstacle is the lack of protocols for efficiently generating therapeutically-sufficient numbers of purified lineage-specific cells. Adding uncertainty to the application of iPSC-derived cells in regenerative medicine is the fact that cells differentiated *in vitro* are less mature than those that develop *in vivo*, and might not be able to integrate into the host tissues upon transplantation. In addition to these problems that all types of pluripotent stem cells have, incomplete reprogramming or genetic aberrations that accrue during iPSC derivation pose issues such as *de novo* immunogenicity and genomic instability. Because of this, even the reliability of an iPSC-based drug screen would not be so solid as to be unchallengeable.

ES age, iPSC decade and the post-iPSC era

Stem cell research promotes novel therapeutic innovations in regenerative medicine, which are an important complement to conventional medical interventions. It is conceivable that pluripotent stem cells such as ESCs and iPSCs, somatic stem cells and functional cells obtained through other approaches would be recognized as key players in regenerative medicine. The late 1990s and early 2000s represent the age of hESCs, as these cells were recognized during those years as offering a great promise both to the scientific community and the public. However, in addition to ethical dilemmas, issues such as researchers' poor understanding of the nature of true pluripotency, risks of tumor formation and immune rejection upon allograft transplantation were not easily solvable and enthusiasm for stem cells started to vanish as the public lost their patience after years of waiting. Nonetheless, the reprogramming of human fibroblasts back to a pluripotent state with only a few transcription factors or small molecules was a great breakthrough in the stem cell field, and launched a new decade of stem cell research. iPSCs provide another important avenue to study pluripotency, and can be used to develop systems for disease modeling, drug discovery and cell-based therapy. iPSCs have some advantages over hESCs such as the absence of ethical concerns and presumably immune rejection issues. Nevertheless, as a new type of stem cell, they still must be studied using hESCs as a reference for a complete understanding of their nature and realization of their application potential. In fact, in the context of cell replacement therapy, hiPSCs are subject to the same requirement that applies to hESCs: they need to be reliably differentiated in large quantities and be functional before they can be used for therapy. Compromising the potential use of iPSCs for cell replacement therapy are the suboptimal procedures for iPSC production, mutations during reprogramming and uncertainties over the genomic stability of the differentiated derivatives. In this regard, other avenues, such as direct lineage conversion or transdifferentiation, might be more promising for personalized regenerative medicine in the future. We expect in a post-iPSC era there will be more advances in concepts and breakthroughs in translational research, addressing reprogramming, pluripotency and cell fate change.

Conclusion

Ever since the use of Oct4, Sox2, Klf4 and c-Myc (OSKM) to reprogram somatic cells into pluripotent stem cells, breakthroughs in the iPSC field have been reported frequently and these advances greatly challenge our conventional understanding of cell fate determination. Pluripotency, which has long been considered as being atop the epigenetic potency valley, is probably a balanced state of counteracting differentiation cues. Pluripotency factors, which were thought to prevent differentiation by inhibiting the action of lineage specifiers, are not indispensable for reprogramming somatic cells back to a pluripotent state. Other lineage specifiers, when employed appropriately, are also able to generate iPSCs. In this regard, pluripotency represents just one of the states among many. iPSCs could be very useful in modeling diseases and screening drugs, and clarification of the molecular mechanism of reprogramming and cell fate determination is also important to efficiently produce the desired specific type of cells for cell-based replacement therapy. However, as a type of pluripotent stem cells still needing further investigation, iPSCs are not likely ideal for this purpose. Other techniques that are being developed based on the theory of reprogramming and fate change, such as direct lineage conversion or reprogramming of somatic cells into lineage specified progenitors, might fulfill the promises of personalized cell-based therapy.

Competing interests

The authors declared that no competing interests exist.

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