

# Induced pluripotent stem cells in medicine and biology

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## Summary

Differentiated cells can be reprogrammed to pluripotency and other cell fates by treatment with defined factors. The discovery of induced pluripotent stem cells (iPSCs) has opened up unprecedented opportunities in the pharmaceutical industry, in the clinic and in laboratories. In particular, the medical applications of human iPSCs in disease modeling and stem cell therapy have been progressing rapidly. The ability to induce cell fate conversion is attractive not only for these applications, but also for basic research fields, such as development, cancer, epigenetics and aging.

**Key words:** Disease modeling, Reprogramming, Stem cell, Therapy, iPSCs

## Introduction

All cells that exist in the body, both somatic (of all three germ layers) and germ cells, originate from the pluripotent cells of the embryo. The recently developed induced pluripotent stem cell (iPSC) technology, which enables the induction of pluripotency in mature somatic cells by treatment with defined factors, has created new avenues in basic research, disease modeling and regenerative medicine (Takahashi and Yamanaka, 2006). However, as the proverbial phrase goes, ‘Rome wasn’t built in a day’ and in fact the foundations of this field go back over 50 years. In groundbreaking work, Sir John Gurdon succeeded in generating cloned frogs by transferring the nucleus of a tadpole’s somatic cell into an oocyte (Gurdon, 1962) (Fig. 1A). His remarkable study demonstrated that the oocyte can completely reverse the acquired memories of somatic cells. Before his breakthrough, differentiation had long been considered to be a one-way street, with traffic flowing from an immature state, a stem or progenitor cell, to a more mature differentiated state. This is often depicted by the ‘epigenetic landscape’ proposed by Conrad Waddington (Waddington, 1957) (Fig. 2). In parallel, it was previously believed that unnecessary genetic information was deleted or terminally inactivated in cells committed to a specific state, a theory known as Weismann’s barrier (Weismann, 1893). Nuclear reprogramming demonstrated that not only do somatic cells retain all genetic information, but they can also be rejuvenated by artificial manipulations to again acquire pluripotency.

Another key discovery in scientific history that enabled us to generate iPSCs was a landmark experiment that first demonstrated direct cell fate conversion by a defined transcription factor (Davis et al., 1987) (Fig. 1B). The authors of that study performed complementary DNA subtraction and found three genes that were expressed predominantly in proliferative myoblasts. Enforced expression of one of them, myogenic differentiation 1 (MyoD1), was

alone sufficient to convert mouse fibroblasts to myosin-expressing stable myoblasts. This was the dawn of direct reprogramming, whereby somatic cells are induced to transit directly from one lineage to another. Direct reprogramming is now one of the biggest trends in cell biology research, yielding important new discoveries (Takahashi, 2012).

These separate fields of nuclear transfer and direct reprogramming combine neatly into the concept of induced pluripotency (Fig. 1C). Like nuclear transfer, a state of pluripotency is achieved, and iPSCs bear a striking resemblance to embryonic stem cells (ESCs). However, the technology is taken from the direct reprogramming field via the enforced expression of defined transcription factors. Although the details and mechanisms of the reprogramming process during iPSC generation are still being elucidated, the products are promising for many purposes, such as drug discovery, pathological studies, toxicology studies, the evaluation of secondary drug effects and regenerative medicine, to name a few (Yamanaka, 2009). In this Spotlight article, we outline the current knowledge and highlight future prospects for induced pluripotency.

## Cell transplantation therapy

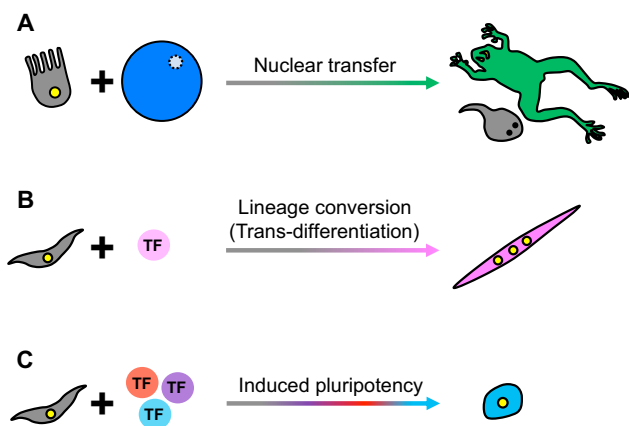
Since the first report in 1998, human ESCs have been expected to be key for the treatment of intractable diseases, such as Parkinson’s disease and spinal cord injury (Thomson et al., 1998). However, the use of human ESCs faces several hurdles, including ethical concerns about using human embryos and scientific concerns about immune rejection after transplantation. iPSC technology has the potential to overcome these issues.

In 2007, the proof of concept of the therapeutic use of iPSCs was reported for the mouse model of sickle-cell anemia, a genetic blood disorder caused by a defect in the  $\beta$ -globin gene (Hanna et al., 2007). Gene correction by homologous recombination in a mutant iPSC line, followed by transplantation into mutant mice, cured the disease. This was a perfect model for iPSC-mediated regenerative medicine: diseases caused by single gene defects can be addressed by made-to-order gene replacement in autologous cells. Compared with allografts from other donors, the iPSC-based autologous method is advantageous in that the risk of immunological rejection, transmission of unidentified viruses or other forms of infection is negligible (Araki et al., 2013; Guha et al., 2013). Realistically, however, using autologous iPSCs from each individual would be associated with high medical costs. In addition, because more than 3 months are needed to generate iPSCs using the current methods, the effective treatment of some disorders, such as spinal cord injury, cannot be achieved within the necessary timeframe.

It is therefore important to consider the use of allogeneic iPSC lines for regenerative medicine. Fortunately, a major advantage of the iPSC technology is the diversity of donor candidates and ease of access to the cells of origin. Before generating clinical-grade iPSC clones, all aspects of the donors, such as their health conditions and the type of human leukocyte antigen (HLA) expressed, can be examined. In addition, based on the information of donors, it is

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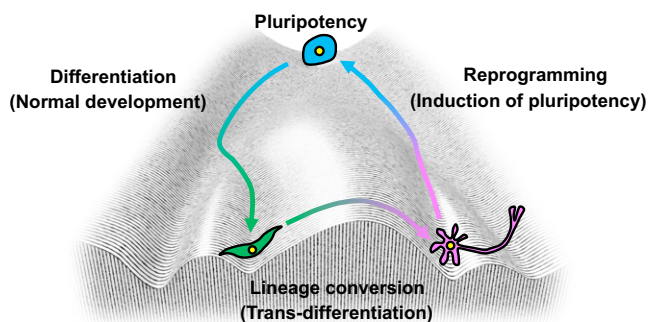
**Fig. 1. Key conceptual advances leading to the discovery of iPSCs.**

(A) Oocyte-mediated reprogramming. The memory of somatic cell nuclei can be erased by injection into enucleated oocyte. The generation of cloned feeding tadpoles by nuclear transfer was achieved in 1962. (B) Transcription factor-induced conversion of lineage committed cell fate. Lineage-instructive transcription factor(s) (TFs) can change somatic cell fate to a destination state beyond germ layers. In some cases, such cell fate conversion can be referred to as trans-differentiation. (C) Induction of pluripotency by defined factors. The combined concepts of oocyte-mediated reprogramming and transcription factor-induced cell fate conversion allowed us to discover the iPSC technology.

possible to determine whether single nucleotide and copy number variations found in iPSC clones already existed in the donor as somatic mosaicism or whether these were newly acquired during iPSC formation. Another strength is the abundant availability of multiple clones from each donor. Human pluripotent stem cells generally show clonal variations in lineage bias, which allows for cells to be chosen that best suit the purposes of each application (Osafune et al., 2008; Kajiwaru et al., 2012). Based on experience with bone marrow transplantation, matching the three major types of HLA loci, including A, B and DR, between the recipient and donor is expected to elicit less immune rejection after transplantation. Thus, at present, the most realistic method for iPSC therapy is based on the collection of iPSC stocks derived from various HLA-homozygous donors (Nakajima et al., 2007; Okita et al., 2011).

There were many problems associated with the early human iPSCs, such as the integration of retroviral vectors, which led to a risk of tumorigenicity, and the requirement for undefined serum-containing media to support iPSC generation. However, many of the issues have been resolved by recent technological innovations, such as the use of episomal plasmids that do not integrate into the genome, and xeno-free culture (Yu et al., 2009; Chen et al., 2011; Okita et al., 2011; Okita et al., 2013). We expect that, in the near future, it will be possible to generate clinical-grade iPSCs under Good Manufacturing Practice (GMP) compliance.

A distinctive characteristic of iPSC technology is the diversity of their sources. Human iPSCs should ideally be obtained using minimally invasive procedures associated with the lowest possible risk. Human iPSCs were first established from dermal fibroblasts (Takahashi et al., 2007; Yu et al., 2007). A skin biopsy for fibroblast collection is a relatively minor concern for individuals who would otherwise require multiple surgeries or high-risk drug treatments. For healthy individuals, a biopsy may leave a visible scar and there is always some risk of infection, and therefore the harvest site must be selected with caution.



**Fig. 2. Cell fate changes on Waddington's epigenetic landscape.**

Pluripotent stem cells can be differentiated into various somatic lineages not only in response to extrinsic cues *in vitro* but also during development. Recent technologies with tissue-specific transcription factors enable the conversion of lineage committed cells to another fate. A small set of reprogramming factors also reverts differentiated cells to pluripotency. Adapted, with permission, from Waddington (Waddington, 1957).

Cord blood is an attractive source for iPSCs (Giorgetti et al., 2009; Haase et al., 2009; Takenaka et al., 2009). Immature cells, abundant in cord blood, may be a suitable source not only because of their availability, but also because they may have acquired fewer genetic mutations than adult-derived cells. On the other hand, although cord blood banks should stock only the cells derived from seemingly healthy donors, it is difficult to trace whether the donors remain healthy when they are older.

Peripheral blood is another promising source of iPSCs. In particular, T-lymphocytes can be reproducibly converted to iPSCs by using transient expression methods (Okita et al., 2013; Seki et al., 2011). Because the rearrangement patterns of T-cell receptor loci are different in each T-lymphocyte, and are readily distinguishable, it would be easy to monitor and trace the clonality of iPSCs under GMP. Furthermore, it is possible to confirm the health status of a donor by medical diagnosis and patient history, at least up until their present age. Because of the minimal invasiveness of venipuncture, the cooperation of many healthy donors is expected. There are large stocks of cord blood and donor blood candidates in many nations, and, as such, blood represents one of most powerful potential sources of clinical grade iPSCs available.

### Disease modeling and drug screening

A common belief is that cell transplantation therapy represents the best medical application of iPSCs. In our opinion, disease modeling and drug screening are at least as important as the applications for cell therapy (Yamanaka, 2010). Animal models have contributed tremendously to a better understanding of disease mechanisms. However, there are limitations to the use of animal models in terms of an accurate recapitulation of human diseases. For example, a number of drugs have been developed that showed therapeutic effects in rodent models of amyotrophic lateral sclerosis (ALS). Unfortunately, all of them turned out to be ineffective in human patients, emphasizing the necessity of disease models using human cells (Desnuelle et al., 2001; Shefner et al., 2004).

Disease-specific iPSC lines were first reported by two groups in 2008 (Dimos et al., 2008; Park et al., 2008), and *in vitro* reconstruction of the disease state was first successful in the case of spinal muscular atrophy (SMA) (Ebert et al., 2009; Ebert et al., 2012). These studies showed not only the possibility of reproducing disease phenotypes by using patient-derived iPSCs, but also the

potential applications of using these cells for drug screening. To date, many patient-specific iPSC lines have been established and used for disease modeling, and are expected to facilitate studies on rare diseases (Bellin et al., 2012). One of the crucial issues regarding patient-derived iPSCs is an adequate control. Although ESCs and iPSCs derived from healthy donors are readily available, the major differences in the genetic backgrounds are associated with controversies regarding their use for comparisons with diseased cells. Cells from healthy family members such as mothers and brothers are better controls for comparison, because they have fewer genetic differences from the cells taken from diseased donors. Recent progress in genetic editing technologies using zinc-finger nucleases and transcription activator-like effector nucleases have made gene correction in patient-specific iPSC lines more realistic (Hockemeyer et al., 2009; Hockemeyer et al., 2011).

### Applications in the basic sciences

A small set of core transcription factors, called reprogramming factors, trigger the destruction of the existing state of somatic cells by changing their epigenetic status, leading to alterations in their gene expression. The changes in the balance of gene expression may induce secondary epigenetic changes, including DNA methylation and alterations of the nuclear architecture. During such stepwise changes in somatic cell fate, it is necessary to eliminate a number of roadblocks (Vierbuchen and Wernig, 2012). These roadblocks to reprogramming serve as barriers that ensure the stability of cellular identity, but core pluripotency transcription factors such as OCT3/4 and SOX2 are able to destabilize the existing order in the original cells and re-construct a new order. Therefore, transcription factors associated with development may undertake important actions during the reprogramming process. Although NANOG is also a core member of the pluripotency transcription network, it is dispensable for the generation of iPSCs (Chambers et al., 2003; Mitsui et al., 2003; Takahashi and Yamanaka, 2006; Takahashi et al., 2007). The function of NANOG in the pluripotency network may therefore be distinct from that of OCT3/4 and SOX2, at least in the context of reprogramming. These important studies highlight the overlap between reprogramming technologies and developmental biology.

The advent of reprogramming technologies has helped to elucidate the relationship between epigenetic modification and cellular identity, although our understanding here is still incomplete. Reprogramming techniques can be used to dissect the role of transcriptional and epigenetic changes in cancer development. The process of reprogramming towards pluripotency shares some common events with carcinogenesis, such as mesenchymal-epithelial transitions and the involvement of cancer-related proteins such as Myc and p53 (Bernhardt et al., 2012). *In vitro* aging studies using reprogramming technology are also an interesting and challenging area of research. Reprogramming somatic cells to iPSCs allows us to obtain 'rejuvenated' cells that can provide a tool with which to investigate the major causes of aging, and the accumulation of genetic abnormalities or epigenetic errors that occur over time.

### Emerging technologies

Mimicking developmental processes *in vitro* by treatment of iPSC cultures with extrinsic factors such as cytokines, and specific structural and microenvironment niches, has already been used to generate cerebral cortex, retina and pituitary gland tissues with appropriate three-dimensional structures (Eiraku et al., 2008; Eiraku et al., 2011; Suga et al., 2011). In another example of generating tissues, this time *in vivo*, injection of wild-type rat pluripotent stem

cells into blastocysts of *Pdx1* deficient mice – which are unable to form a pancreas – resulted in the generation of normally functioning rat pancreatic tissue (Kobayashi et al., 2010). Self-organization of tissue development would be valuable not only for the next generation of organ transplantation, but also for disease modeling, and is a major advantage of pluripotency-mediated strategies.

The success of germ cell differentiation from mouse pluripotent stem cells is a recent significant advance in the pluripotent stem cell field (Hayashi et al., 2011; Hayashi et al., 2012). Pluripotent stem cell-derived primordial germ cells (PGC) open a new avenue for infertility studies. As mouse epiblast-derived stem cells (EpiSC) cannot differentiate into PGCs, naive pluripotency, which is a ground state of mouse ESCs reflected by the features of the inner cell mass, is required for PGC differentiation. Human pluripotent stem cells show characteristics of mouse EpiSCs rather than naive mouse ESCs, and so they probably have less potential for PGC differentiation (Brons et al., 2007; Tesar et al., 2007; Greber et al., 2010). The generation of bona fide naive human pluripotent stem cells may allow for human PGC differentiation (Buecker et al., 2010; Hanna et al., 2010; Wang et al., 2011).

Recapitulating the differentiation process from beginning to end *in vitro* is often hard because of the insufficient understanding of the molecular mechanisms, including the epigenetic changes that occur during directed differentiation. Directed differentiation is time-consuming, and the resulting cells may often represent embryonic cell types. In the case of humans, maturation to adult cell types might require the full length of the typical gestational period, or even longer. This problem may be relieved somewhat by using direct reprogramming methods, which force somatic cells to undergo conversion to another lineage while bypassing pluripotency (Takahashi, 2012; Vierbuchen and Wernig, 2012). Furthermore, direct reprogramming has been shown to rapidly generate mature differentiated cells (Caiazzo et al., 2011; Pfisterer et al., 2011; Son et al., 2011), which are likely to better recapitulate late-onset disease phenotypes.

By contrast, the benefits of reprogramming-mediated rejuvenation have been demonstrated in the quality of T-lymphocytes derived from iPSCs (Nishimura et al., 2013; Vizcardo et al., 2013). The antigen specificity of T cells is irreversibly determined by the T-cell receptor gene-rearrangement patterns. Therefore, antigen-specific T cells can be expanded via reprogramming to iPSCs followed by re-differentiation. This approach not only solved the limitations associated with the number of cells needed for immunotherapy but also achieved rejuvenation as a by-product of reprogramming, as the reprogrammed cells had renewed proliferative capacity and telomere elongation upon re-differentiation into T cells.

### Conclusions

In this Spotlight article, we have summarized some of the potential applications of iPSCs and emerging related technologies. These include applications in basic biology, as well as cellular therapeutics and disease modeling. Combined with other emerging technologies, such as epigenomic profiling and deep sequencing, we predict a bright future for iPSC technology in both the clinic and the laboratory.

### Acknowledgements

We are grateful to Yoko Miyake, Rie Kato, Saki Okamoto, Eri Minamitani, Sayaka Takeshima and Ryoko Fujiwara for their valuable administrative support, and to all of our present and former colleagues, including alumni, for their encouragement and sharing data. We apologize for any data that we were unable to cite because of space limitations.

**Funding**

This work was supported in part by a grant from the Program for Promotion of Fundamental Studies in Health Sciences of National Institute of Biomedical Innovation, a grant from the Leading Project of Ministry of Education, Culture, Sports, Science and Technology (MEXT), a grant from the Funding Program for World-Leading Innovative Research and Development on Science and Technology (FIRST) of the Japan Society for the Promotion of Science, Grants-in-Aid for Scientific Research of the Japan Society for the Promotion of Science and MEXT.

**Competing interests statement**

S.Y. is a member without salary of the scientific advisory boards of iPierian, iPSC Academia Japan, Megakaryon Corporation and Retina Institute Japan.

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