# Induction of Pluripotency by Defined Factors

SHINYA YAMANAKA

*Center for iPS Cell Research and Application (CiRA)*
*Kyoto University*

## ABSTRACT

Induced pluripotent stem cells (iPSCs) were originally generated from mouse and human skin fibroblasts by introducing 4 transcription factor genes. iPSCs are similar to embryonic stem cells (ESCs), having a potential to produce cells for all the tissue types in the body such as neuron, blood, eyes and heart. iPSCs can be generated from various genetically identified individuals including patients. These iPSCs and subsequently differentiated target cells/tissues would provide unprecedented opportunities in regenerative medicine, disease modelling, drug screening, and proof-of-concept studies in drug development.

Compared to ESCs, iPSCs have less ethical controversy since they can be generated without destroying fertilized eggs. However, as iPSCs still share some ethical issues with ESCs, it is necessary to deepen the discussion in order to make further progress on iPSC research.

Induced pluripotent stem cells (iPSCs) were originally generated from mouse and human fibroblasts via the retroviral introduction of *Oct3/4*, *Sox2*, *c-Myc* and *Klf4* (Takahashi and Yamanaka 2006: 663–76, Takahashi et al. 2007: 861–72). The iPSCs are similar to embryonic stem cells (ESCs) in terms of their morphology, gene expression, and most importantly, pluripotency and self-renewal.

Compared to ESCs, iPSCs can be generated from various genetically identified individuals, including patients with diseases for which there is no appropriate animal model, or those with specific human leukocyte antigen (HLA) types. Patient-specific

iPSCs provide unprecedented opportunities for disease research, drug screening and toxicology studies. A stock of iPSC clones constructed from HLA homozygous donors would therefore provide a significant resource for cell therapy.

For the future clinical application of iPSC technology, there are several technical hurdles that must be overcome to ensure the safety of iPSCs as a source of cell therapies. Recent reports on tumour formation following transplantation and the large diversity between iPSC clones highlight some of the potential problems.

One concern that was highlighted as a potential problem is the possibility of tumour formation caused by the reactivation of retroviral integrated genes in iPSCs. As alternatives to retroviral transduction, several alternative protocols for iPSC generation have been proposed, such as using lentiviruses, adenoviruses, plasmids, transposons, recombinant proteins, synthetic mRNA or chemical compounds. Our research team has also reported an integration-free induction method using episomal vectors (Okita et al. 2011: 409–12). This method can induce human iPSCs efficiently and reproducibly. Regarding the iPSC induction factors, we discovered that L-Myc (Nakagawa et al. 2010: 10.1073) and Glis1 (Maekawa et al. 2011: 225–29) can be used to establish iPSCs with high efficiency and quality, replacing the oncogene c-Myc. Many research groups worldwide have been developing iPSCs induction protocols to further enhance the capability of producing safe and effective cell sources. However, in addition to these technical view points, it is necessary to consider the ethical issues related to further progress of iPSC research.

Human ESCs also have potential for use in regenerative medicine. However, the generation of human ESCs poses ethical and religious issues, as these cells are generated by destroying human embryos left over from *in vitro* fertilisation procedures. In fact, although more than ten years have gone by since human ESCs were first established (Thomson et al. 1998: 1145–47), the development of their applications has been limited. One reason for this lack of progress with ESCs is that multiple restrictions were enacted against studies to generate human ESCs. For example, the former US President George W. Bush prohibited the generation and use of human ESCs with federal research funds after taking office, and the Vatican announced that the medical use of ESCs 'will undermine the human dignity and will not be tolerated from a bioethical viewpoint'.

As human iPSCs are generated from human somatic cells without using embryos, most of the ethical questions can be avoided. However, iPSCs share some ethical issues with ESCs. One example is the generation of germ cells from ES/iPSCs.

Whereas germ cells derived from human ES/iPSCs can be useful to elucidate the mechanisms involved in human infertility, it will also assist reproduction technology. This could lead to the possibility of the birth of a child resulting from fertilisation of the ES/iPSC-derived gametes, thereby provoking debate over the propriety, as well as the safety, of this technology.

In Japan, there are no statutes or regulations governing the use of human embryos for research. Some Japanese guidelines regarding human stem cells were revised or established in 2010 to permit germ cell differentiation only for the purposes of research into the mechanisms underlying development and regeneration, or for the development of diagnostics, preventive, or regulatory medical procedures or products; but fertilisation via gametes derived from human pluripotent stem cells has been prohibited (MEXT 2009, MEXT 2010). Under these guidelines, studies are being undertaken of the molecular profiles and characteristics of germ cells and their role in human germline development.

Another example of an ethical issue shared by both iPSCs and ESCs involves a recently developed iPSC application, which enabled researchers to generate a rat pancreas in a mouse by microinjecting rat iPSCs into mouse blastocyts that were deficient in pancreas development (Kobayashi et al. 2010: 787–99). Using this technology, it might become possible to regenerate healthy human organs *in vivo*. However, this technology is ethically controversial because it means creating a human/animal chimera. In Japan, the research on human-animal chimeric embryos is regulated under the Act on the Regulation of Human Cloning Techniques (Government of Japan 2000), which requires notification of a Cabinet minister, and which prohibits the transfer of the embryos into a uterus and handling the embryos for more than 14 days after fertilisation.

It is expected that the application of research with iPSCs will be accelerated, as iPSCs can be prepared relatively easily. There is a need to deepen the discussion with the public and bioethicists, as scientific technology sometimes advances ahead of ethical acceptability. Researchers should not only follow the present guidelines, but also respond quickly to emerging ethical issues by disclosing as much information about their studies as possible to the public so that the public can understand the research properly and can then make decisions regarding the use of such new technologies.

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