

INDUCED PLURIPOTENT STEM CELLS - QUICK FACTS

Introduction

Induced pluripotent stem cells (iPSCs or iPS cells) refer to pluripotent stem cells that are artificially induced from differentiated cells. The iPSC technology was first developed by the Yamanaka lab at Kyoto University after identifying several genes that are active in embryonic stem cells. Further studies identified four transcription factors (Oct4, Sox2, Klf4 and c-Myc) essential for the production of pluripotent stem cells from differentiated cells. By using retroviral vectors to express these genes, adult mouse fibroblasts were re-programmed into iPSCs that successfully made it to the germ line in the chimeric animals after their injection into mouse blastocysts [1-3]. Subsequent experiments with the iPSC technology were successfully applied to re-program differentiated human cells [4, 5], thereby demonstrating the value of iPSC technology for clinical applications.

Several groups have demonstrated that iPSCs can be derived by forced expression of Oct4 alone [6] or a combination of two or three transcription factors [7], with or without the help of small molecules such as the histone deacetylase inhibitor valproic acid (VPA) [8] or the histone methyltransferase inhibitor BIX-1294 [9]. Success of each of these approaches is dependent on the starting cell populations. For example, neural stem cells that endogenously express Sox2, c-Myc, Klf4, and several intermediate reprogramming markers have been induced into iPSCs with Oct4 alone [9]. To date, iPSCs have been successfully generated from skin cells, stomach cells, liver cells, pancreatic cells, lymphocytes, testis germline and neural stem cells [1-5,10-15,18].

Important Genes Involved in Generating iPSCs

Oct4 (or Oct-3/4): Oct4 is encoded by the gene Pou5f1. As a member of the "Oct" transcription factor family, this 38 kD protein contains a POU domain and a homeobox domain, and binds to the octamer motif (5'-ATTGTCAT-3'). It forms a trimeric complex with Sox2 on DNA and is implicated in regulating the expression of a number of genes such as YES1, FGF4, UTF1, and ZFP206 that are involved in embryonic development. Oct4 is exclusively expressed in pluripotent stem cells, and appears in a diffused and slightly punctuated pattern in the nucleus. A sumoylation at lysine 123 enhances the protein stability, DNA binding, and transactivation activity and is required for YES1 expression enhancement. Several pseudogenes of POU5F1 have been described on chromosomes 1, 3, 8, 10, and 12. Two of them, localized on chromosomes 8 and 10, are transcribed in cancer tissues but not in normal tissues and may be involved in the regulation of POU5F1 gene activity in carcinogenesis [10]. **No iPSCs have been produced without using Oct4.**

Sox2: This 34 kD transcription factor contains a HMG box for DNA binding and forms trimeric complexes with Oct4 and the octamer motif to control the expression of a number of genes. Sox2 is expressed in pluripotent, multipotent, and unipotent stem cells. Interestingly, sumoylation of Sox2 inhibits its binding on DNA and negatively regulates the FGF4 transactivation. Defects in Sox2 are the cause of microphthalmia syndrome type 3, a clinically heterogeneous disorder of eye formation, ranging from reduced eye size to complete bilateral absence of ocular tissues (anophthalmia) [11]. The Sox family of genes is associated with maintaining pluripotency similar to Oct4, although it is also associated with multipotent and unipotent stem cells. Recent reports suggest that Sox2 is not necessary for iPSC induction from neuronal progenitor cells where an endogenous Sox2 expression exists.

Klf4 (Kruessel-like factor 4): Also known as EZF (epithelial zinc finger protein) or GKLf (gut-enriched kruessel-like factor), this 55 kD transcription factor contains three zinc finger domains, a serine-rich region, and a proline-rich region. Klf4 acts as both an activator and repressor of transcription. It binds the CACCC core sequence at multiple sites in the 5' flanking region of its own gene and can activate its own transcription. It interacts with the c-terminal domain of MUC1 to enhance the suppression of TP53/p53 transcription. Although initially identified as a required factor for the generation of iPSCs, Thompson *et al* reported that Klf4 was unnecessary for generating human iPS cells, and in fact failed to generate them. Huangfu *et al* reported that in the presence of histone deacetylase inhibitor VPA, it is possible to produce iPSCs without Klf4, but the induction efficiency was enhanced one-thousand fold with Klf4. Klf4 is not required for inducing iPSC from neural stem cells that express Klf4 endogenously [9].

Nanog and LIN28: Nanog is a 35 kD transcription factor that is involved in ES cell proliferation and self-renewal. In embryonic stem cells, Nanog, Oct4, and Sox2 co-occupy promoters of several hundred genes, suggesting they work coordinately or as a complex in promoting pluripotency [10]. Lin28 is an mRNA binding protein expressed in embryonic stem cells. However, neither Nanog nor Lin28 was necessary for iPSC production in many cases, although the Thomson lab has reported they can be used together to replace Klf4 and c-Myc to generate iPSCs.

c-Myc: In early reports, the oncogene c-myc was implicated in the generation of mouse iPSCs. It is now known that c-myc was only an enhancing, not a necessary, factor for the development of iPSCs.

Gene Delivery Approaches for Generating iPSCs

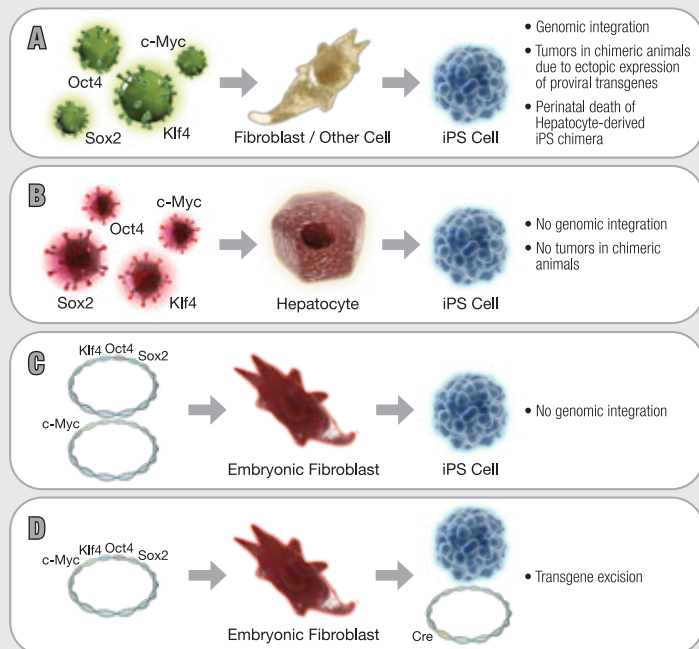


Figure 1: Generating iPSCs. Gene delivery methods for generating iPSCs [Retroviral or Lentiviral Transduction (A), Adenoviral Transduction (B), Plasmid Transfection (C), Transposon-based Transfection (D)].

There are many ways to deliver these key transcription factors into target cells for generating iPSCs. The first generation of iPSC technologies used retrovirus or lentivirus transduction (Figure 1A) for delivery. The immediate safety concern about this approach is the genome integration of virus DNA, which could potentially result in altered differentiation potential or other malignant transformation. Later it was shown that transient expression of the key transcription factors by adenoviral vectors can also induce iPSC (Figure 1B). The advantage of adenovirus vector based expression is that the transgenes will not integrate into the house genome, thus reduces the risk of tumorigenesis. A plasmid based transfection can avoid the genome integration too, but the iPSC induction rate is lower (Figure 1C). Recently, the Cre-recombinase excisable systems are reported to be efficient in iPSC induction and subsequent transgene removal ([13-15] and Figure 1D), making the iPSC technology one step closer to clinic applications. Other delivery methods such as protein transduction [16] are promising safer alternatives.

How to Characterize iPSCs?

Induced pluripotent stem cells are believed to be identical to natural embryonic stem cells in many aspects, including colony morphology (sharp-edged, tightly packed) and growth properties (self-renewing, proliferation, and dividing rate).

The most important property of iPSCs is its pluripotency to differentiate into any cell type. Continued progress of iPSC technologies for research studies, and potential expansion of iPSC technologies for clinical applications, is reliant on reliable methods to test the pluripotency of cells. Recent articles on criteria and standards to assess the iPSCs provide great insights and perspectives [17-19].

The most stringent method to evaluate the pluripotency of iPSCs is to inject the ES cells into host blastocysts to assay if viable chimeras are produced (Figure 2A). By using an appropriate reporter system or cell markers, cells from the iPSC lineage can be traced throughout the whole body of chimeric mice. However, chimera formation is not a practical approach for human cells, nor even for mouse cells, as this is time-consuming and not applicable to most research laboratories.

A less stringent method to assess the pluripotency of iPSCs is teratoma formation assay. In this assay, iPSCs injected under the skin of nude mice should develop teratomas with the distinct structure of all three germ layers (ectoderm, mesoderm, and endoderm). The teratoma assay requires a threshold level of cells to prove successful, though the exact number has not yet been identified.

The more frequently used method for assessing the pluripotency of iPSCs is to differentiate *in vitro* the putative iPSCs into various cell types and assay for cell markers (Figure 2B). These include gene expression markers, antigen markers, and epigenetic markers (DNA methylation and histone modification patterns). For human iPSCs, frequently used stem cell expression markers are AP1, Nanog, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, and TRA-2-49/6E. For mouse iPSCs, AP1, Nanog, and SSEA-1 are often used. Human and mouse iPSCs have been differentiated *in vitro* into neural stem cells, neurons, cardiomyocytes, etc. Specific expression and antigen markers are available for each of these cell types.

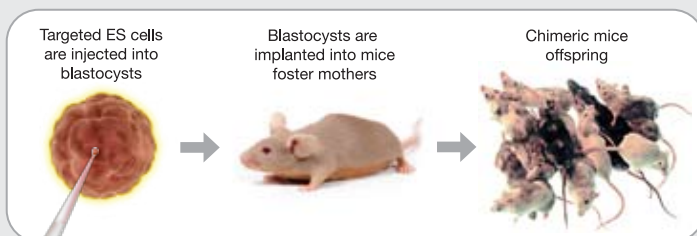


Figure 2A: Evaluation Model for Pluripotency. Evaluation of iPSCs by the chimera formation

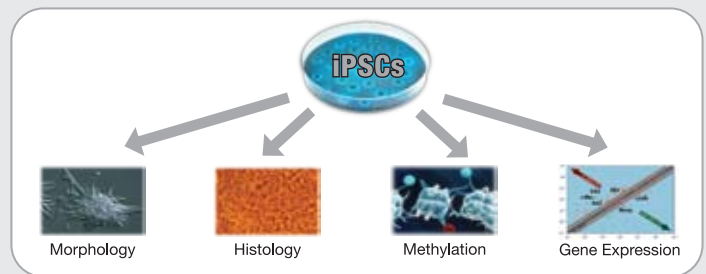


Figure 2B: Evaluation Model for Pluripotency. Molecular biology analysis.

Milestones of iPSC Technology

August 2006: Induced pluripotent stem cells were first generated by Yamanaka's team at Kyoto University, Japan [1].

June 2007: The Yamanaka lab, Hochedlinger lab and Jaenisch lab successfully re-programmed mouse fibroblasts into iPSCs and even producing viable chimera [2, 3].

November 2007: The Thomson lab and the Yamanaka lab created iPSCs from adult human cells [4, 5].

December 2007: The Jaenisch lab successfully treated transgenic mice carrying the human gene for sickle-cell anemia by introducing hematopoietic stem cells derived from those mice's gene-repaired iPSCs [11].

August 2008: iPSCs generated from an 82-year-old woman with amyotrophic lateral sclerosis (ALS) were differentiated into motor neurons, the cell type destroyed in ALS [12].

September 2008: Konrad Hochedlinger and colleagues successfully used an adenovirus to transport the requisite four genes into the DNA of skin and liver cells of mice, resulting in cells identical to embryonic stem cells [10].

October 2008: The Yamanaka lab demonstrated reprogramming can be accomplished via plasmid-based methods [11].

November 2008: iPSC was generated from human testis germline [18].

February 2009: iPSC was induced from adult neural stem cells with one factor Oct4 [6].

March 2009: The Cre-recombinase excisable based system that removes the transgenes after iPSC generation was successfully employed [13-15].

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