

Review on Induced Pluripotent Stem Cell and its Potential Clinical Applications

Abstract

The generation of induced pluripotent stem cell (iPSC) from somatic cells demonstrated that mature mammalian cells can be reprogrammed to a pluripotent state by the enforced expression of few embryonic transcription factor. iPSCs are reprogrammed from human somatic cells through ectopic expression of various transcription factors viz. Oct4, Sox2, Klf4, and c-Myc (OSKM). This novel technology enables derivation of patient specific cells, which possess a potential cure for many diseases. In addition, iPSC technology has provided researchers with a unique tool to derive disease-specific stem cells for the study and possible treatment of cancer and also the degenerative disorders with autologous cells. Many cancer cells exchange with these cells and cure the cancer. We summarize in this article the potential clinical application of iPSC.

Keywords: iPSC; Embryonic stem cell; Embryonic transcription factors; Cancer

Review Article

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Abbreviations: NK: Natural Killer; AML: Acute Myeloid Leukemia; CSE: Cancer Stem Cell; CML: Chronic Myeloid Leukemia; JMML: Juvenile Myelomonocytic Leukemia; FPD: Familial Platelet Disorder; CAMT: Congenital Amegakaryocytic Thrombocytopenia; SCN: Severe Congenital Neutropenia; OCT4: Octamer Binding Transcription Factor 4; IVF: *In Vitro* Fertilization; iPSC: Induced Pluripotent Stem Cell

Introduction

Stem cells are the creation cells for every organ in our bodies. The very highly differentiated cells that make up these tissues originally came from the starting pool of stem cells formed shortly after fertilization. We continue to rely on stem cells to change injured tissues and cells that are lost every day, such as those in our skin, hair, blood and the lining of our gut. Stem cells have two key characteristics:

- The capability to identify, regenerate, splitting in a way that makes duplicate of themselves and
- The ability to distinguish, giving rise to the full grown types of cells that makes up our organs.

Embryonic stem cells have been obtained from a variety of species, including humans, and are described as “pluripotent,” meaning that they can create all the non-identical types of cells in the body. Embryonic stem cells can be obtained from the blastocyst, a very early phase growth that consists of a mostly void ball of approximately 150-200 cells and is barely seen to the eye. At this phase, there are no organs, not blood, just an “inner cell mass” from which embryonic stem cells can be produced. Human embryonic stem cells are obtained initially from blastocysts that were generated by *in vitro* fertilization (IVF) for assisted reproduction but were no longer needed. The fertilized egg and

the cells that instantly appear in the few divisions are “totipotent.”

Induced Pluri Potent Stem Cell

These are mature cells (e.g., skin cells) that are engineered, or “reprogrammed,” to become pluripotent, i.e., behave like an embryonic stem cell. While these iPS cells share most of the similar properties of embryonic stem cells, including the capability to give rise to all the cell types in the body, it is important to understand that they are not similar. The actual iPS cells were generated by using viruses to insert extra copies of three to four genes known to be important in embryonic stem cells into the differentiated cell. It is not yet completely understood how these three to four “Reprogramming” genes are able to induce pluripotency; this question is the focus of ongoing research. In addition, recent studies have focused on alternative ways of reprogramming cells using methods that are safer for use in clinical settings (Figure 1).

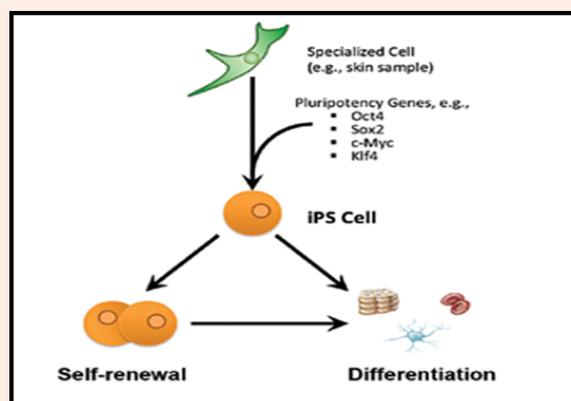


Figure 1: Formation of iPSC.

Gene Used in Induced Pluripotent Stem Cell

Oct family

It is first of the family of octamer transcription factors, and plays a leading role in maintaining pluripotency. The lack of Oct-3/4 in Oct-3/4⁺ cells, such as blastomeres and embryonic stem cell, leads to voluntary trophoblast distinction, and presence of Oct-3/4 thus gives rise to the pluripotency and differentiation potential of embryonic stem cells. Some other genes in the "Oct" family, including Oct-3/4's close relatives, Oct1 and Oct6, fail to elicit induction, thus demonstrating the exclusiveness of Oct-3/4 to the induction process.

Sox family

The Sox family of transcription factors is related with maintaining pluripotency same to Oct-3/4, while it is related with multipotent and unipotent stem cells in difference with Oct-3/4, which is expressed in pluripotent stem cells. Sox1 produces iPSC cells with a similar regulation as Sox2, and genes Sox3, Sox15, and Sox18 also produce iPSC cells, although with reduce efficiency.

Klf family

Klf4 of the Klf family of transcription factors was first identified by Yamanaka et al. [1] and confirmed by Jaenisch et al. [2] as a factor for the generation of mouse iPSC cells and was demonstrated by Yamanaka et al. [1] as a factor for generation of human iPSC cells. However, Thomson et al. [3] reported that Klf4 was needless for production of human iPSC cells and in fact stop to generate human iPSC cells. Klf2 and Klf4 were found to be factors capable of generating iPSC cells, and equivalent genes Klf1 and Klf5 did as well, although with less efficiency.

Myc family

The Myc family of transcription factors are proto oncogenes incriminated in cancer. Yamanaka et al. [1] and Jaenisch et al. [2] demonstrated that c-myc is a factor implicated in the generation of mouse iPSC cells and Yamanaka et al. [1] demonstrated it was a factor implicated in the generation of human iPSC cells. However, Thomson & Yamanaka et al. [1,2] Usage of the "myc" family of genes in induction of iPSC cells is worry for the case of iPSC cells as clinical therapies, as 25% of mice transplanted with c-myc-induced iPSC cells developed lethal teratomas. N-Myc and L-Myc have been identified to induce instead of c-myc with same efficiency.

Nanog

In embryonic stem cells, Nanog, along with Oct-3/4 and Sox2, is compulsory in promoting pluripotency. Hence, it was surprising when Yamanaka et al. [1] described that Nanog was unwanted for induction while Thomson et al. [2] has described it is possible to generate iPSC cells with Nanog as one of the factors.

LIN28

LIN28 is an mRNA binding protein expressed in embryonic stem cells and embryonic carcinoma cells related with differentiation and proliferation. Thomson et al. [1] demonstrated that LIN28 is a factor in iPSC generation in combination with OCT4, SOX2, and NANOG.

Glis 1

Glis1 is transcription factor that can be used with Oct-3/4, Sox2 and Klf4 to induce pluripotency. It poses numerous advantages when used instead of C-myc.

Clinical Application of iPSC

iPSCs are acquired through the reprogramming of a particular somatic stem cells by the introduction of certain transcription factors. Their chief value is based on their pluripotency to differentiate into cells of all three germ layers, which makes them a useful tool for the discovery of new drugs and the formation of cell therapy programs.

iPSC technology makes it, to develop patient-specific cell therapy protocols as they are genetically identical to the donor and thus prevent the occurrence of an immune rejection in autologous transplantations. Also, dissimilar embryonic stem cells, they are not associated with any ethical controversies and therefore regulatory conditions for their use are much less stringent (Table 1 & 2).

Therapeutic potential of iPSC

In addition to being an inspiring research tool to probe mammalian development and epigenetic reprogramming, iPSCs have therapeutic potential for both custom-tailored cell therapy and so-called "disease modeling." These two concepts are illustrated in Figure 2.

Organ transplantation between nonrelated individuals is complicated by the limited availability of matched tissues and the requirement for life-long treatment with immunosuppressive drugs that can have side effects. iPSCs might circumvent these problems, as they could be coaxed into the wanted cell types that would be genetically matched with the patient. Another key benefit of iPSCs over current transplantation approaches is the possibility of repairing disease-causing mutations by homologous recombination, a technology that has been used with success in mature stem cells because of notorious difficulties in growing them outside the body.

iPSCs in cancer

Severe congenital neutropenia (SCN): The autosomal recessive type of SCN is caused by lack of HAX1 gene. iPSC lines were generated from a patient with SCN having HAX1 gene deficiency. Genetic correction of iPSCs was made by a novel *in vitro* neutrophil differentiation system. The study resulted in advance of faulty granulopoiesis.

Congenital amegakaryocytic thrombocytopenia (CAMT): Myeloproliferative leukemia virus oncogene (MPL), encoding for thrombopoietin receptor, is nonfunctional in patients with CAMT. CAMT is caused by deficiency of thrombopoietin-related MPL-mediated signaling which induces pancytopenia causing to bone marrow failure with the onset of thrombocytopenia and anemia prior to leucopenia. Generated CAMT-iPSCs exhibited faulty MPL signaling. Excessive MPL signaling in both normal and CAMT-iPSCs led to damaging megakaryopoiesis and production of CD41a+, CD42a- and CD42b- megakaryocytes and platelets.

Myelofibrosis: iPSCs were successfully generated from patients with:

- a. Major myelofibrosis with chromosome 13 deletion and
- b. Secondary myelofibrosis with JAK 2 V617 F mutation.

These disease specific iPSCs provide a research tool for studying the disease and potentially providing targeted therapy.

Myelodysplasia and acute myeloid leukemia (AML)

MonoMAC syndrome is caused by GATA 2 deficiency and patients are predisposed to myelodysplastic syndrome and AML transformation. In a pre-clinical study, iPSCs were obtained from a

patient with GATA 2 syndrome harboring R361H mutation. These iPSCs displayed severe depletions in hematopoietic differentiation potential and absent clonogenic capacity. Familial platelet disorder (FPD) is an autosomal dominant disease of hematopoietic system that is caused by heterogenous mutations in RUNX1. In study, iPSC lines were generated from fibroblasts of a patient with FPD. After correction of RUNX1 mutation, megakaryopoiesis was restored. In a third pre-clinical study, iPSCs were established from 3 distinct FPD/AML pedigrees. These FPD-iPSCs were shown to be uniformly defective in emergence of hematopoietic progenitor cells and megakaryocytic differentiation. In addition, the phenotypes of FPD-iPSCs were found to be a consequence of haploinsufficiency of RUNX1 [4-6].

Table 1: Clinical Application of iPSCs.

1. Disease modeling.	
2. Cell-based therapies.	
3. Synthesis of blood components: the following blood components have been generated from embryonic and iPSCs:	
a. Red blood cells [definitive and primitive erythroid cells]; can be used in severe anemia or blood loss.	
b. Megakaryocytes and platelets; transfusion in critical thrombocytopenia.	
c. Natural killer cells: natural or antibody – assisted anticancer cytotoxicity.	
d. Neutrophils.	
4. Regenerative medicine: tissue engineering and organ repair.	
5. Drug screening for toxicity, drug development and drug discovery.	
6. Genetic therapy: treatment of intractable and genetic disorders:	
Sickle cell anemia	Spinal muscular atrophy
Retinitis pigmentosa	Cystic Fibrosis
beta- Thalassemia	Lysch- Nyhan syndrome
Hemophilia	Hurler syndrome
Fanconi anemia	Down's syndrome
Dyskeratosis congenita	Schizophrenia
Schwachman-Bodian-Diamond Syndrome	Alzheimer's disease
Primary myelofibrosis	Pompe disease

Table 2: Models of diseases treated with iPSC-based interventions.

Disease Condition	Therapeutic Outcome Achieved
Sickle cell diseases	Improvement of hematopoiesis at functional and physiological levels.
Hemophilia A	Survival benefit obtained Decreased clotting time achieved.
Parkinson's diseases	Dopamine production enhanced, leading to symptomatic improvement.
Ischemic heart diseases	In situ tissue repair leading to improved cardiac performance.

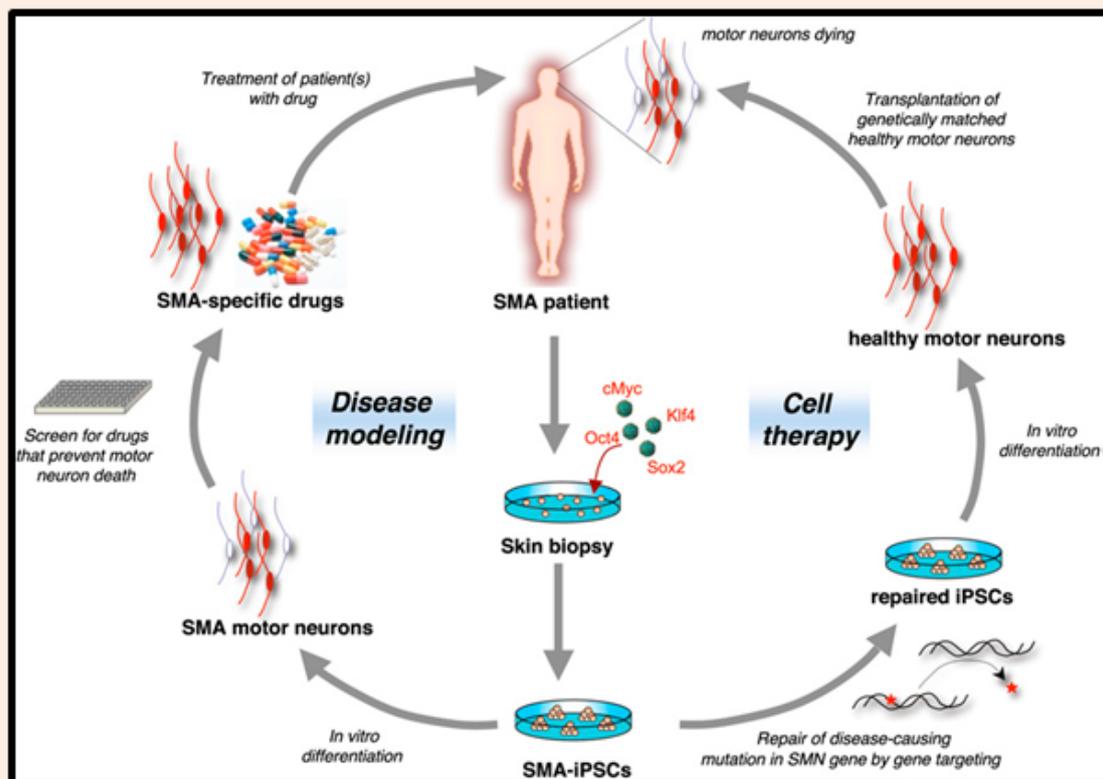


Figure 2: Potential applications of iPSCs in cell therapy.

Juvenile myelomonocytic leukemia (JMML)

iPSCs were produced from malignant cells belonging to 2 children with JMML having p.E76K mutations in the PTPN11 gene. Pharmacological inhibition of MEK (PD0325901) kinase in iPSCs-derived JMML cells caused normalization of granulocyte monocyte-colony stimulating factor (GM-CSF) independence and hypersensitivity in myeloid precursors of JMML-iPSCs. These results provide a rationale for a targeted therapy for JMML.

Chronic myeloid leukemia

iPSCs were generated from primary CML patient samples. The methylation pattern and the gene expression of CML-iPSCs were similar to those with normal iPSCs. CML-iPSCs provide a platform to investigate CML pathogenesis on the basis of patient derived samples.

Lymphomas

The concept of cancer stem cell (CSC) in AML has provided an understanding of carcinogenesis and relapses. These leukemia-originating stem cells are critical for the initiation of leukemias but, the existence of a same cell population that may generate B-cell lymphocytic malignancies remains uncertain. Detailed selection and molecular characterization of the specific cells of origin of each B-cell lymphoma entity are essential steps to better understanding of lymphomagenesis and to develop effective and

potentially curative therapeutic modalities.

Solid tumors

iPSCs have also been generated from malignant cells belonging to patients with gastrointestinal cancers. Antitumor immunotherapy using T cell and natural killer (NK) cell-based therapies has demonstrated promising therapeutic potentials in patients with renal cell carcinoma, malignant melanoma and chemotherapy refractory AML. The ability to modify human ESC and iPSC-derived NK cells with tumor-specific receptors may be utilized against a wide range of malignancies in the near future after having the appropriate pre-clinical and clinical trials performed. Cancer-derived iPSCs are expected to provide a novel experimental opportunity to establish disease models.

Conclusion

iPSC are pluripotent cells that can be derived from various cell lines and can be reprogrammed to give rise to any cell type found in the body. Their clinical applications are rapidly expanding and these include: cell therapy, genetic therapy and disease therapy. The most significant of these cells to the field of cell therapy are related to the treatment of such organ specific condition such as immunological disorders, many cancer and hematological diseases. Importantly, iPSC could overcome the problem of immune rejection.

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