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Induced Pluripotent Stem Cells: Origins and Directions

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Abstract

Embryonic stem (ES) cells are used in various fields for diverse purposes, including gene targeting, cell therapy, tissue repair, organ regeneration, and so on. However, studies on and applications of ES cells are hindered by ethical disputes regarding cell source. To circumvent ethical issues, scientists have attempted to generate ES cell-like cells, which are not derived from the inner cell mass of blastocyst-stage embryos. In 2006, Yamanaka first reprogrammed mouse embryonic fibroblasts into ES cell-like cells, which were called induced pluripotent stem (iPS) cells. Nearly a year later, the Yamanaka and Thomson laboratories independently reprogrammed human somatic cells into iPS cells. Since the establishment of the first iPS cell line, iPS cells have been derived from a number of different cell types and have been used for cell therapy, human disease modeling, and drug discovery. The use of peripheral blood facilitates research on iPS cells and enables the establishment of patient-specific iPS cells. With the improvement in iPS cell technology, clinical therapy based on iPS cells will rapidly develop.

Keywords: Induced pluripotent stem cells; Origin; Peripheral blood; Differentiation; Application; Potential issues

Introduction

Embryonic stem (ES) cells are totipotent cells derived from the inner cell mass of blastocyst-stage embryos. Totipotency is the potential to differentiate into various kinds of cells, such as muscle cells, neural cells, and even germ cells. Due to this property, ES cells can be used to generate any type of cell to meet the requirements of different applications. ES cells are also capable of self-renewal; they can be semi-permanently cultured using feeder cells, which provide them with necessary growth factors.

In 1981, mouse ES cells were established by Evans and Kaufman [1]. At present, mouse ES cells are widely used to generate gene-targeted animals. These animal models have made great contributions to basic research on gene functions. Unfortunately, although human ES cells were established back in 1998 [2], research on these cells and their clinical application have been restricted by ethical disputes regarding cell source and immunological rejection in cell therapy. Most debates on ethics primarily focus on the morality of destroying human embryos for the benefit of other people. Obtaining stem cell lines from oocytes and embryos is fraught with disputes regarding the onset of personhood and reproduction. Moreover, ES cells from different donors have different immunizing antigens. Somatic cells differentiated from human ES cells and transplanted into a recipient may be rejected by the recipient's immune system. Therefore, making patient-specific pluripotent stem cells is necessary.

To develop a new kind of stem cell with self-renewal properties and pluripotency, scientists have tried to reprogram somatic cells through many methods, such as nuclear transfer [3,4], cell fusion [5], and so on. In 2006, a breakthrough was made by a Japanese group. Yamanaka Laboratory generated induced pluripotent stem (iPS) cells by over-expressing a few types of transcription factors. In this review, we focus on the origins, differentiation, and applications of iPS cells. In addition, we provide a discussion on the potential issues on and future perspectives for iPS cells.

The origin of iPS Cells

The nuclei of mouse somatic cells can be reprogrammed if they are hybridized with mouse ES cells. The hybridized somatic cells were observed to be capable of differentiating into endoderm, mesoderm, and ectoderm cells. These findings demonstrated that reprogramming factors, which are expressed in ES cells, could induce pluripotency in somatic cells. The most difficult part in reprogramming somatic cells is finding these reprogramming factors which can convert somatic cells to pluripotent stem cells.

How to solve this problem?

Fbx15 is a gene expressed specifically in ES cells. Normal fibroblasts cannot survive in the presence of Geneticin (G418), an analog of Neomycin (Neo) used for screening ES cells. Therefore, fibroblasts with a Neo resistance gene in their Fbx15 locus are used to screen for candidate reprogramming factors. Fibroblast reprogramming by the candidate reprogramming factors activates the Fbx15 locus, which leads to the expression of Neo resistance genes, thereby allowing the fibroblasts to survive in the presence of G418.

In Yamanaka's experiments, 24 genes, which were important transcripts of ES cells and oncogenes, were selected as candidate reprogramming factors. Different combinations of these candidates were introduced into mouse embryonic fibroblasts using the Fbx15-Neo reporter system; G418-resistant mouse stem cell-like colonies would appear about two weeks later if these candidate genes could reprogram the fibroblasts. The 24 candidates were finally narrowed down to four transcription factor genes. Using the retroviral-mediated factors Oct3/4, Sox2, Klf4, and c-Myc, Yamanaka was able to reprogram mouse embryonic fibroblasts into ES cell-like cells in

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2006 [6]. These reprogrammed cells were named iPS cells. This was a revolutionary breakthrough that immediately sparked immense interest. However, the four transcription factor genes did not fully activate some key pluripotency genes; hence, the fibroblasts were only partially reprogrammed. When the iPS cells were injected into mouse blastocysts, they failed to generate postnatal chimeras or contribute to the germline. In 2007, totipotent iPS cell lines were established; live chimeras and germline transmitted mice were generated from these iPS cells though blastocyst injection [7-9]. In 2009, Zhou [10] and Gao [11] used iPS cells to produce germline transmitted mice through tetraploid complementation. Research in different laboratories, as mentioned above; indicate that iPS cells, similar to ES cells, have the potential to differentiate into any cell type.

Nearly a year after the breakthrough, scientists from the Yamanaka [12] and Thomson [13] laboratories independently reprogrammed human somatic cells to iPS cells. The former used Oct3/4, Sox2, Klf4, and c-Myc on human dermal fibroblasts, whereas the latter used Oct3/4, Sox2, Nanog, and Lin28 on human somatic cells. Both undertakings demonstrated that human iPS cells resemble human ES cells in many aspects, including morphology, proliferation, pluripotency markers, gene expression profiles, epigenetic status, and differentiation potential. These findings revealed that human iPS cells have the potential to replace human ES cells. Human iPS cells address the ethical disputes over stem cell sources and immunological rejection in cell therapy.

Since the first iPS cell line was established in 2006, advances have been made to improve the safety and efficiency of the reprogramming process, such as single [14] and multiple transient transfections [15], non-integrating vectors [16-18], excisable vectors [19-21], direct protein transduction [22-24], RNA-based Sendai viruses [25-27], mRNA-based transcription factor delivery [28,29], microRNA transfections [30], and the use of chemical compounds [31,32]. In addition, various cell sources now also facilitate research on iPS cells. Up to the present, iPS cells have been derived from a number of different species, including mouse, human, rat, marmoset, rhesus monkey, pig, rabbit, and so on (Table 1). However, most iPS cell lines fail to yield live chimeras. With regard to cell type, iPS cells have been generated from fibroblasts, hepatocytes, gastric epithelial cells, keratinocytes, mesenchymal cells, neural stem cells, pancreatic cells, B and T lymphocytes, blood progenitor cells, cord blood cells, peripheral blood cells, and so on (Table 1).

iPS cells from peripheral blood

Generating patient-specific iPS cells is a critical step in cell therapy and other clinical applications. As shown in Table 1, human

Species	Cell type	Factors or Chemicals	Vectors	References
Mouse	Fibroblast	OKSM or OKS	retrovirus	[6,33,34]
	Fibroblast	OSE or KSNr	retrovirus	[35,36]
	Fibroblast	mir302/367 cluster	lentivirus	[37]
	Fibroblast	OKSM	PB transposon and 2A peptides	[19]
	Fibroblast	proteins (OKSM)	poly-arginine	[23]
	Fibroblast	OKSM	plasmid or adenovirus	[15,16]
	Dermal papilla	OKM or OK	retrovirus	[38]
	Melanocyte	OKM	drug-inducible lentivirus	[39]
	Mature B and T cell	OKSM	retrovirus	[40]
	Myeloid progenitor	OKSM	retrovirus	[40]
	Hematopoietic stem cell	OKSM	retrovirus	[40]
	Pancreatic β cell	OKSM	drug-inducible lentivirus	[41]
	Intestinal epithelial cell	OKSM	drug-inducible lentivirus	[42]
	Hepatocyte	OKS	retrovirus	[43]
	Gastric epithelial cell	OKSM	retrovirus	[43]
	Adipose stem cell	OKSM	retrovirus	[44]
	Neural stem cell	OK or O	retrovirus	[45,46]
Human	Fibroblast	OKSM or OKS	retrovirus	[12,33]
	Fibroblast	OSLN	lentivirus	[13]
	Fibroblast	OKSM or OKS	floxed lentivirus	[47]
	Fibroblast	OS and valproic acid	retrovirus	[48]
	Fibroblast	proteins (OKSM)	poly-arginine	[22]
	Fibroblast	OKSM	adenovirus	[49]
	HUVEC	OKSM	retrovirus	[50]
	Peripheral blood cell	OKSM	drug-inducible lentivirus	[51,52]
	Cord blood endothelial cell	OSLN	lentivirus	[53]
	Cord blood stem cell	OKSM or OS	retrovirus	[40,54]
	Adipose stem cell	OKSM	lentivirus	[55]
	Adipose stem cell	OKS	retrovirus	[56]
	Amniotic cell	OKSM	retrovirus	[57]
	Amniotic cell	OSN	lentivirus	[58]
	Neural stem cell	O	retrovirus	[59]
	Marrow mesenchymal cell	OKSM or OK	retrovirus	[60]
	Adipose stem cell	OSLN	nonviral minicircle DNA	[60]
	Hepatocyte	OKSM	retrovirus	[62]
	Astrocyte	OKSM	retrovirus	[63]
	Keratinocyte	OKSM or OKS	retrovirus	[64]
Pig	Fibroblast	OKSM	drug-inducible lentivirus	[65]
Rabbit	Hepatocyte and stomach cell	OKSM	lentivirus	[66]
Rat	Fibroblast	OKS	retrovirus	[67]
	Fibroblast	OKSM	lentivirus	[68]
	Neural progenitor cell	OKS	retrovirus	[67]
	Liver progenitor cell	OKS	retrovirus	[69]
Marmoset	Fibroblast	OKSM	retrovirus	[70]
Rhesus monkey	Fibroblast	OKSM	retrovirus	[71]

Abbreviations: O: Oct3/4; S: Sox2; K: Klf4; M: c-Myc; E: Esrrb; L: Lin28; N: Nanog; Nr: Nr52a.

Table 1. iPS Cells Derived from Different Species and Somatic Cell Types

iPS cells are most commonly derived from dermal fibroblasts, due to their accessibility and relatively high reprogramming efficiency. However, dermal fibroblasts require skin biopsy and a prolonged period of expansion in cell culture prior to use. During skin biopsy, the exposure of the dermis to ultraviolet light might increase the risk for chromosomal aberrations. In addition, the pain experienced by and risk of infection for patients in obtaining dermal fibroblasts cannot be ignored. These concerns limit the wide application of iPS cells.

Peripheral blood is the most easily accessible source of patient tissue for reprogramming without the need to maintain cell cultures extensively prior to reprogramming experiments. Furthermore, numerous peripheral blood samples have already been frozen and stored in blood banks, and these may be used to generate human iPS cells.

The reprogramming of peripheral blood cells began with research on mice in 2008. Hanna et al. [72] utilized retroviral-mediated factors (Oct3/4, Sox2, Klf4, and c-Myc) to reprogram mouse B lymphocytes; in their experiments, the reprogramming efficiency was improved by either ectopic expression of the myeloid transcription factor CCAAT/enhancer-binding-protein-alpha (C/EBPalpha) or knockdown of the B cell transcription factor Pax5. In 2009, Hong reported the generation of iPS cells from mouse T lymphocytes by the introduction of Oct3/4, Sox2, Klf4, and c-Myc in a p53-null background [73].

After reprogramming mouse peripheral blood cells, Haase generated human iPS cells from cord blood (CB) in 2009 [53]. CB may be obtained from public and commercial CB banks without any risk to donors. In the same year, Ye derived human iPS cells from previously frozen CB and $CD34^+$ cells of healthy adult donors [74]. However, CB is still not easily accessible because it can only be obtained from neonates.

In 2010, three laboratories [26,51,52] independently reprogrammed human peripheral blood cells into iPS cells. In Loh's laboratory, they isolated mononuclear cells (PBMCs) and CD 34^+ cells ($PBCD34^+$) from peripheral blood samples, collected though venipuncture, via Ficoll density centrifugation. After infection with lentiviruses expressing Oct3/4, Sox2, Klf4 and c-Myc, $PBCD34^+$ cells showed a reprogramming efficiency of 0.002%, whereas PBMCs showed relatively lower values of 0.0008% to 0.001% [51]. Starek et al. [52] used a doxycycline-inducible lentivirus construct to derive iPS cells from T lymphocytes and myeloid cells cultured in IL-7 or G-CSF, GM-CSF, IL-3, and IL-6; this lentivirus construct could encode four reprogramming factors (Oct3/4, Sox2, Klf4 and c-Myc) into a polycistronic expression cassette (pHAGE2-TetOminiCMV-hSTEMCCA). Their results showed that the efficiency of reprogramming T lymphocytes was higher compared with that of myeloid cells, this was because T lymphocytes exhibited a higher proliferation rate and had better long-term growth potential in vitro than myeloid cells. Seki induced T lymphocytes into iPS cells using a temperature-sensitive mutant Sendai virus (SeV) vector encoding human Oct3/4, Sox2, Klf4, and c-Myc with an efficiency of 0.1%. This SeV vector is a non-integrating type, and it could not proliferate at standard culture temperatures; these characteristics significantly increase the safety of the generated iPS cells [26]. In 2011, Chou reprogrammed newborn CB and adult peripheral blood mononuclear cells into iPS cells with an improved EBNA1/OriP plasmid. Using this new reprogramming vector, iPS cells were generated from peripheral blood cells within 14 days, instead of 28 to 30 days as in a previous work on fibroblasts [75].

The research and findings described above indicate that human iPS cells from peripheral blood cells are comparable to human ES cells in terms of morphology, surface antigens, pluripotency gene expression, DNA methylation, and differentiation potential: induced pluripotent stem cells from mononuclear cells of peripheral blood can be considered reliable. Hence, methods of generating iPS cells from human peripheral blood cells will accelerate research on and promote future clinical applications of iPS cells.

Differentiation and applications of iPS cells

Similar to ES cells, mouse iPS cells have the potential to differentiate into any type of cell, and even the capability of germline transmission [7-11]. This means that we can obtain differentiated cells in large quantities. So far, iPS cells have main applications in three major areas: cell therapy, human disease modeling, and drug discovery.

Cell therapy: Immunological rejection is a major problem in organ transplantation and cell therapy, and long-term treatment with immunosuppressive drugs has serious side effects. Patient-specific iPS cells have the immune markers of the patient, so they address the problem of immunological rejection. In addition, disease-causing mutations can be repaired by gene targeting in patient-specific iPS cells. Repaired cells can be differentiated into targeted cells and transplanted into the diseased area where they can alleviate disease symptoms. To illustrate this, using a mouse model, Jaenisch and his colleagues showed that iPS cells can be used to cure sickle cell anemia, a genetic blood disorder that renders red blood cells nonfunctional. The disease-causing mutation was repaired in iPS cells derived from the mouse model via gene targeting. The repaired iPS cells were then differentiated into blood-forming progenitor cells. These healthy progenitors were transplanted into an anemic mouse where they generated normal red blood cells, thereby curing the disease [76].

Disease modeling: For many human genetic diseases, therapeutic research is hindered by issues regarding the source of experimental materials. iPS cells can overcome these problems by establishing patient-specific disease models. Patient-specific iPS cells can form cell lineages that reflect the defects caused by a certain disease in patients. Some human diseases for which models have been established using patient-specific iPS cells include amyotrophic lateral sclerosis [77], spinal muscular atrophy [78], Parkinson's disease [47], β-thalassemia [79], Rett syndrome [80], adenosine deaminase deficiency-related severe combined immunodeficiency, Shwachman-Bodian-Diamond syndrome, Gaucher disease, Duchenne muscular dystrophy, Becker muscular dystrophy, Huntington's disease, type 1 diabetes mellitus, Down syndrome, and Lesch-Nyhan syndrome (carriers) [60].

Drug discovery: Before using novel drugs for treatment, reliable data on their potential toxic effects on humans must be determined. In drug discovery, the effects and side effects of novel drugs are usually tested in laboratory animals. However, these tests are costly, and humans and animals have relatively significant differences. In addition, animal tests are not effectively standardized. Novel drugs can be tested efficiently on disease models generated from patient-specific iPS cells. This approach will greatly facilitate research on pharmacology and toxicology. Some drugs have already been tested on iPS cells derived from patients suffering from various diseases, including spinal muscular atrophy [78], familial dysautonomia [81], and LEOPARD syndrome [82]. Observations that novel drugs alleviate "symptoms" in patient-specific iPS cells demonstrate their therapeutic potential. This

principle can now be applied to many other diseases and will benefit many patients.

Potential Issues Regarding iPS Cells

Crucial experiments based on iPS cell technology have shed light on human diseases at the cellular and molecular level. The application of iPS cells in drug discovery can reduce cost and increase the chance of success. Furthermore, iPS cells circumvent ethical disputes on ES cells, and patient-specific iPS cells may resolve problems of immunological rejection in cell therapy.

Currently, in the field of iPS cells, scientists are developing more efficient methods of deriving iPS cells from various cell sources, including those from patients who suffer from different diseases. More progress and new innovations regarding iPS cells are to be made in the near future. However, some problems remain to be solved in the clinical application of iPS cells. Various kinds of genomic changes, including chromosomal aneuploidy, translocations, point mutations, megabase-scale duplications and/or deletions, and so on, have been observed in human iPS cells; these problems may affect the therapeutic potential of iPS cells. Although the reprogramming process itself might cause chromosomal anomalies, not all anomalies result from it, as genomic alterations have been identified in human iPS cells produced through different techniques, including non-integrating methods such as those that use synthetic mRNAs [29]. Nevertheless, compared with improving reprogramming efficiency, solving problems in chromosomal anomalies in iPS cells is more important. Thus, in the future, the focus of the field of iPS cells should be shifted toward obtaining iPS cells with the fewest genomic alterations. The causes of chromosomal abnormalities during iPS cell induction need to be investigated as well.

In addition, some other questions remain: What is the mechanism of iPS cell induction? What are the optimal reprogramming factors? How do we reduce risks of insertion mutagenesis in the genome of iPS cells? How do we achieve directed differentiation? How do we evaluate the safeness of iPS cells in clinical applications? Obtaining the answers to these questions require thorough analyses of the induction process and the epigenetics of iPS cells. Moreover, a reliable evaluation system on clinical trials needs to be established.

Future Perspectives

The generation of iPS cells is regarded as a milestone for life science. Despite the problems mentioned in last section, the advantages of using iPS cells cannot be ignored. With iPS cells, ethical disputes can be avoided, as well as immunological rejection in cell therapy, using patient-specific iPS cells. Moreover, disease models generated using iPS cells can be used to study the mechanism of human genetic disorders and test the effects of novel drugs. iPS cell biology has admittedly become a new field within stem cell research that covers various important and attractive scientific areas.

A more comprehensive knowledge of the reprogramming process is crucial for future clinical applications of iPS cells. Recent advancements have increased the therapeutic potential of iPS cells. Along with the improvement of iPS cell technology, clinical therapy based on iPS cells will be put on the agenda in the foreseeable future.

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