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REVIEW

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Cell reprogramming for the creation of patient-specific pluripotent stem cells by defined factors

Abstract Pluripotent stem cells (PSCs), characterized by being able to differentiate into various types of cells, are generally regarded as the most promising sources for cell replacement therapies. However, as typical PSCs, embryonic stem cells (ESCs) are still far away from human clinics so far due to ethical issues and immune rejection response. One way to avoid such problems is to use stem cells derived from autologous somatic cells. Up to date, PSCs could be obtained by reprogramming somatic cells to pluripotent state with approaches including somatic cell nuclear transfer (SCNT), fusion with stem cells, coculture with cells' extracts, and induction with defined factors. Among these, through reprogramming somatic cells directly by retroviral transduction of transcription factors, induced pluripotent stem (iPS) cells have been successfully generated in both mouse and human recently. These iPS cells shared similar morphology and growth properties to ESCs, could express ESCs marker genes, and could produce adult or germline-competent chimaeras and differentiate into a variety of cell types, including germ cells. Moreover, with iPS technique, patient specific PSCs could be derived more easily from handful somatic cells in human without immune rejection responses innately connected to ESCs. Consequently, generation of iPS cells would be of great help to further understand disease mechanisms, drug screening, and cell transplantation therapies as well. In summary, the recent progress in the study of cell reprogramming for the creation of patient-specific pluripotent stem cells, some existing problems, and research perspectives were suggested.

Keywords somatic cells, pluripotent stem cells, iPS cells, reprogramming

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1 Introduction

Stem cells are characterized to self-renew and differentiate into a wide variety of cell types. Recent advances of the clinical application of stem cells in bone marrow transplant or dermatology successfully lay a solid basis for the further development of regenerative medicine. Although great progress has been made in these fields, the future of stem-cell-based therapies still depends on two key factors: obtaining suitable stem cell sources and developing efficient technologies for the production of appropriate stem-cell derivatives *in vitro* (Klimanskaya et al., 2008). Therefore, it is important to establish reliable sources of human stem cells for clinical applications. However, we still have not obtained an ideal stem cell candidate for regenerative medicine at present. Since the average organ (e.g., heart and liver) contains several types of cells, the most valuable for regeneration would be pluripotent stem cells.

Human ESCs, as one of PSCs, might be used to treat various neurological, endocrinological, and hematopoietic disorders. Moreover, little success has been achieved in laboratory animals yet not much has been achieved in humans. One problem is that harvesting ESCs from human blastocysts is controversial. The other major problem is immune rejection response. One way to solve the latter problem is to use stem cells that are genetically identical to the host. At present, there are four methods that could directly reprogram somatic cells toward pluripotency, including somatic cell nuclear transfer (SCNT), fusion

with stem cells, coculture with cells' extracts, and induction with defined factors, respectively (Fig. 1).

2 Somatic cell nuclear transfer (SCNT)

SCNT, namely, animal cloning, denotes that a somatic nucleus can be transplanted into an enucleated oocyte and reprogrammed by the competent cytoplasm and also reverse the differentiated genome to its pluripotent state. Usually, SCNT is classified into two types: reproductive cloning and therapeutic cloning. When transferred into a female recipient, a cloned embryo may develop into a fetus (reproductive cloning) or be explanted into culture, which can give rise to ESCs with the same genome as the nuclear donor (therapeutic cloning). Therefore, therapeutic cloning, through the production of these autologous nuclear-transfer embryonic stem cells (ntESC), offers great promises for regenerative and reproductive medicine in gene therapy, as a vector for gene delivery. In this regard, therapeutic cloning in the context of cell replacement therapy holds a huge potential for *de novo* organogenesis, which means that the permanent treatment of neurodegenerative diseases and diabetes mellitus as shown by *in vivo* studies and in the cure of genetic disorders when used in conjunction with gene therapy, also including the use of SCNT product as a vector for gene delivery to establish animal models of human diseases (Kfoury, 2007). Furthermore, SCNT might, in the future, allow *in vitro* organogenesis and counteract senescence.

However, scientific roadblocks impeding advancement in therapeutic cloning include (1) potential abnormalities, (2) mitochondrial heteroplasmy, and (3) ethical issues. Much of previous studies showed that transferred donor cell nuclei always encounter incorrect or incomplete reprogramming. For example, the epigenetic memory of the donor nuclei was frozen during subsequent embryonic development (Smith et al., 2005), which would frequently result in gene dysregulation and defects in the first generation of cloned animals (Kohda et al., 2005). Since ESCs generated by SCNT still included the genes encoded by the mitochondria of oocyte, they might produce minor histocompatibility complex (mHC) differences between donor and host. Human ESCs express mHC, and although they are not as immunogeneic as MHC and ABO antigens, they can nonetheless induce immune rejection (Grinnemo et al., 2008). Therapeutic cloning is also often tied to ethical considerations concerning the source, destruction, and moral status of IVF embryos based on the argument of potential. In addition, a major roadblock in the feasibility of human therapeutic cloning is the low availability of oocytes for research purposes.

3 Fusion with stem cells

Fusion of differentiated cells with pluripotent cells would more or less result in some cell hybrids showing many features similar to PSCs. During the early 1970s, a series of studies indicated that cell fusion could induce reactivation

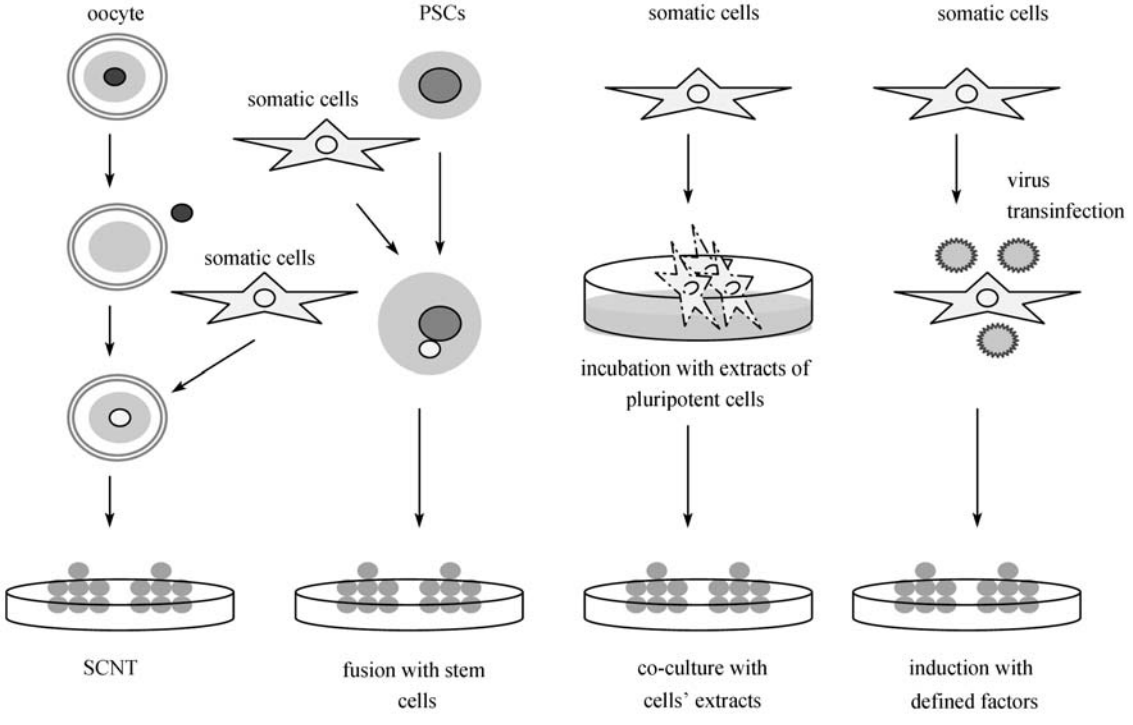

Fig. 1 Four strategies to induce somatic cells to pluripotency

of cell genes. Fusion of pluripotent teratocarcinoma cells with primary thymocytes resulted in the formation of pluripotent hybrids, sharing much features with the parental ECCs (Miller and Ruddle, 1976). The dominance of pluripotent cells over the differentiated cells was also shown in the cell hybrids made between somatic cells/ murine embryonic germ cells (EGCs) (Tada et al., 1997) and somatic cells/ ESCs (Tada et al., 2003), and this reprogramming potential also seemed to be conserved in human ESCs (Cowan et al., 2005). Although ESCs could reset some aspects of epigenetic reprogramming in differentiated cell nuclei, it seems at present that the ability to fully reprogram the epigenome in differentiated cells nuclei is restricted to EGCs (Collas and Taranger, 2006a). Little is known about the molecular mechanisms underlying reprogramming by fusion with stem cells. The fusions between ESCs and neural stem (NS) cells can increase levels of Nanog stimulate pluripotent gene activation from the somatic cell genome. Nanog also improves hybrid yield when thymocytes or fibroblasts are fused to ES cells (Silva et al., 2006).

However, this technology would result in tetraploid cells containing the genome of pluripotent cells (Cowan et al., 2005), fused cells are not fully free from immune rejection after transplanting because of ES cell-derived chromosomes carrying the major histocompatibility complex (MHC) loci. Therefore, it is unlikely to apply this technique to therapeutic purposes. Although Matsumura and colleagues recently have developed a system to remove a selected chromosome from hybrid cells, removing all of the ES cell-derived chromosomes would be technically challenging (Matsumura et al., 2007).

4 Coculture with cells' extracts

Incubation of mammalian somatic cells with extracts of oocytes or pluripotent cells can make nuclei of somatic cells partially reprogrammed to an embryonic state. Human somatic cells exposed directly to extracts of *Xenopus oocytes* can be reversed to some extent in some aspects of cell differentiation (Hansis et al., 2004). Notably, the extracts of embryonal carcinoma cells (ECCs) or ESCs were shown to elicit a shift in the transcriptional program of target cells to upregulate ESC genes and downregulate somatic cell-specific markers and epigenetically modify histones (Collas and Taranger, 2006b). For example, STO and NIH-3T3 mouse fibroblasts were reprogrammed to *Oct-4* and *Rex-1* gene expression and alkaline phosphatase activity by ESCs extracts (Neri et al., 2007). Furthermore, when human somatic 293T cells were permeabilized and incubated in extracts of mouse ESCs, the expression of pluripotency genes (*Oct4*, *Sox2*, *c-Myc* and *Klf4*) was induced rapidly (Bru et al., 2008).

However, application of this technology to produce therapeutic replacement cells still requires further study and development. Because the approach is involved in extensive cell manipulation, the phenotype and genotype of the reprogrammed cells need to be characterized to ensure no genetic perturbations resulted from the process (Collas et al., 2006). In addition, the pluripotent cells generated by this method show no stable reprogramming during subsequent passage culture (Hochedlinger and Jaenisch, 2006).

5 Induction with defined factors

The successful production of ESCs by SCNT, together with the activation of pluripotent genes in somatic–PSCs hybrids and somatic cells exposed to extracts of PSCs indicates that oocytes and pluripotent cells contain factors able to induce reprogramming. Based on this point, in 2006, an infusive study by Takahashi & Yamanaka's laboratory (Takahashi and Yamanaka, 2006) showed that when manipulated to transiently express four transcription factors (Oct4, Sox2, Kfl4 and Myc) by retroviral-mediated transfection and then culture under ESCs conditions, mouse fibroblasts could be reversed to ESCs-like state and termed induced pluripotent stem (iPS) cells. Subsequently, with the scientific discoveries, several studies published not only replicated but also extended the first Takahashi & Yamanaka's findings. Notably, for only a year later, human iPS cells were acquired successfully by introducing four transcription factors into fibroblasts (Takahashi et al., 2007b; Yu et al., 2007; Lowry et al., 2008). These iPS cells were similar to human ESCs in morphology, proliferation, differentiation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity. In addition, genomic DNA analysis suggested genetic origin of iPS cells was truly from their parental fibroblasts, rather than rare stem/ progenitor cells coexisted in fibroblasts cultures. Considering that generation of human iPS cells could hopefully provide a more easily accessible and cost-effective method to create patient- and disease-specific PSCs, scientists regarded it to be invaluable for disease and drug research and, potentially would usher in an era of regenerative medicine (Park and Daley, 2007).

At the very start, Takahashi & Yamanaka selected 24 pluripotency-associated genes as candidates to induce pluripotency in mouse embryonic fibroblasts (MEFs) (Takahashi and Yamanaka, 2006). The ESCs-like colonies were obtained when these candidate factors were reduced by exclusion of single factor stepwise to only four genes: *Sox2*, *Oct3/4*, *Klf4* and *c-Myc*. While Thomson laboratory utilized transcription factor *Oct3/4*, *Sox2*, *Nanog* and *Lin28*, which were tested in a group of 14 candidates, and the results showed *Lin28* gene was not absolutely required for reprogramming and stable expansion of iPS cells, but could influence the frequency of reprogramming (Yu et al., 2007). In addition, iPS cells could also be induced by three

factors with the absence of oncogene *c-Myc* in mouse and human fibroblasts (Nakagawa et al., 2008; Wernig et al., 2008a). However, the efficiency of generating iPS cells was dramatically reduced and the process appeared much more slowly. Accordingly, the efficiency of generating human iPS cells by a combination of six transcription factors could be enhanced (Liao et al., 2008; Mali et al., 2008).

The protocol of generating mouse iPS cells initially was outlined by Takahashi and Yamananaka in the laboratory (Takahashi et al., 2007a). First, a system in which introduction of pluripotency can be detected as marker gene expression was developed. Second, the four genes into somatic cells by retrovirus-mediated transfection were introduced and then cells were under neomycin selection (or using doxycycline-controlled lentiviral vectors system) for reactivation of marker gene, then cultured in ESCs medium. Third, resultant ESCs-like colonies were subsequently analyzed functionally, genetically, and epigenetically to assess to what degree they behaved like ESCs, although generation of iPS cells was a gradual and slow process.

In virtue of different selection marker, the results showed perceptible discrimination (Fig. 2). The selection marker *Fbx15*, which was a downstream gene of *Oct4*, specifically expressed in ESCs and early embryos, but it was dispensable to mouse development and maintenance of pluripotency (Tokuzawa et al., 2003). Fbx15-selected iPS cells exhibited morphology and growth properties similar to ESCs and expressed many good pluripotency marker genes although some were lower than ESCs. Epigenetic changes included a decrease in dimethylation of histone 3 lysine 9 in the *Oct4* and *Nanog* promoter regions, as well as an increase in histone 3 acetylation. Nevertheless, CpG islands in the *Oct4* and *Nanog* regions remained methylated in comparison to ESCs. When transplanted into blastocysts, iPS cells only gave rise to chimeric embryos (Takahashi and Yamanaka, 2006). These data indicated that reprogramming in Fbx15-selected iPS cells was incomplete. It may be correlated with the reactivation of *Fbx15* gene before other pluripotency genes, such as *Nanog*, *Oct4* (Stadtfeld et al., 2008a). To improve the quality of iPS cells, subsequently, a more stringent marker *Nanog* (Maherali et al., 2007; Okita et al., 2007; Wernig et al., 2007) or *Oct4* (Wernig et al., 2007) was selected. Nanog-selected iPS cells expressed most ESCs marker genes at higher and more consistent levels, and the transgene expression was largely silenced compared with Fbx15-selected iPS cells. Especially, undifferentiated iPS cells showed a high level of endogenous Oct4 expression and complete absence of transgene expression. Furthermore, Nanog-selected iPS cells could produce adult germline-competent chimaeras and differentiate into numerous cell types, including germ cells. Female iPS cells represented reactivation of a stochastic silenced X chromosome and underwent random X inactivation upon differentiation (Maherali et al., 2007; Stadtfeld et al., 2008a). In addition, *Oct4* could also be used as a stringent selection marker. Oct4-selected iPS cells were almost indistinguishable from ESCs in the global gene expression and epigenetic pattern and were able to generate viable chimaeras. Compared with *Nanog* as a selection marker, transduction of transcription factors generated significantly more drug-resistant cells from Nanog-neo than from Oct4-neo fibroblasts, but a high fraction of Oct4-selected iPS cells had all the characteristics of pluripotent ESCs (Wernig et al., 2007). In addition, in view of the growth advantage of pluripotent

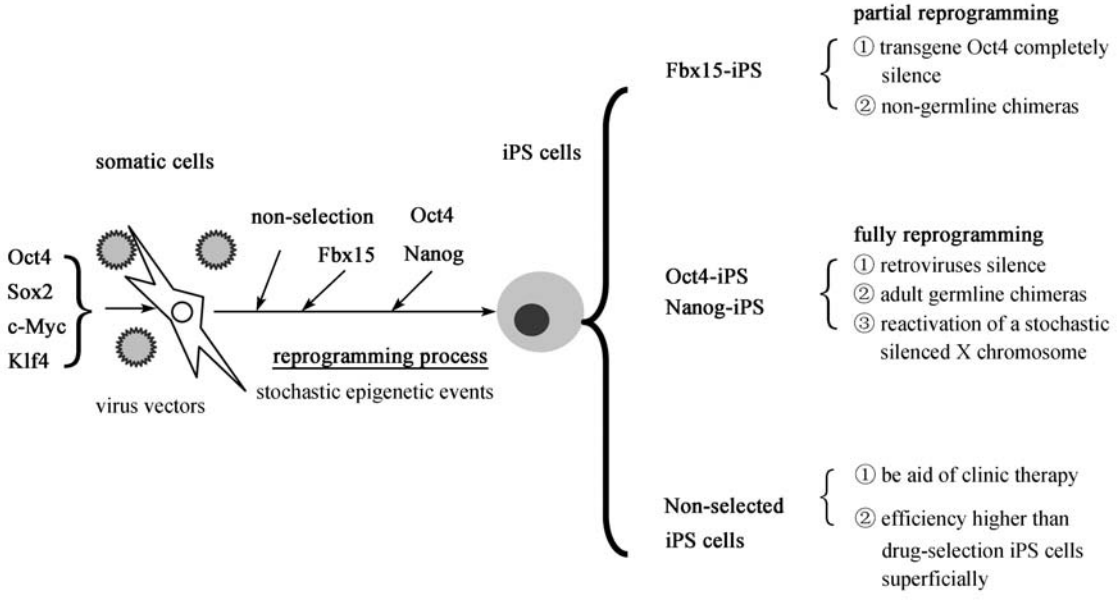

Fig. 2 Reprogramming somatic cells to iPS cells by virus vectors with four transcription factors

cells over the somatic cells, the iPS cells could be isolated from genetically unmodified somatic donor cells solely based on morphological criteria (Blelloch et al., 2007; Maherali et al., 2007; Meissner et al., 2007; Qin et al., 2007). Collectively, these data demonstrated that iPS cells could be achieved by expression of the four factors and using an appropriate selection procedure. Although the selection marker was different, the efficiency of iPS cells generation was very low and less than 1%. Moreover, the efficiency without drug selection seemed higher than gene marker selection based on drug resistance selection superficially.

From the sources of donor cells, the iPS cells were mainly induced from mouse and human fibroblasts. To acquire human iPS cells from adult human somatic cells, additional factors such as hTERT and SV40 might be added up to counterwork apoptosis (Park et al., 2008). In addition, adding SV40 large T antigen (T) to either set of the previously used four reprogramming genes (*Oct3/4*, *Sox2*, *Klf4* and *c-Myc* or *Oct3/4, Sox2,* and *Nanog* and *Lin28*) can enhance the efficiency by 23–70 folds from both human adult and fetal fibroblasts (Mali et al., 2008). Besides fibroblasts, the iPS cells also could be induced from adult mouse hepatocytes and gastric epithelial cells (Aoi et al., 2008). As far as terminally differentiated mouse mature B lymphocytes were concerned, reprogramming to pluripotency required additional overexpression of the myeloid transcription factor CCAAT/enhancer-binding-protein-alpha (C/EBPα) or special knockdown of the Pax5 transcription factor (Hanna et al., 2008). Interestingly, terminally differentiated pancreatic beta cells can also be reprogrammed into iPS cells only by inducible lentiviruses to express four factors (*Oct4, Sox2, c-Myc,* and *Klf4*) (Stadtfeld et al., 2008b). Considering that adult mouse neural stem cells express higher endogenous levels of Sox2 and c-Myc than ESCs, Kim et al. (2008) report exogenous Oct4 only together with either Klf4 or c-Myc is sufficient to generate iPS cells from neural stem cells. Therefore, it is supposed that as long as somatic cells expressed appropriate defined factors, they can be directly reprogrammed into iPS cells.

From the therapeutic potential of iPS cells, the anemia mice could be rescued by transplantation with hematopoietic progenitors obtained *in vitro* from autologous iPS cells by using a humanized sickle cell anemia mouse model (Hanna et al., 2007). In addition, neurons derived from iPS cells could be functionally integrated into the fetal brain and improve symptoms in a rat model of Parkinson's disease upon transplantation (Wernig et al., 2008b). These results demonstrated the therapeutic potential of iPS cells for cell replacement in the animal model. Moreover, the latest studies demonstrate iPS cells can be produced directly from elderly an 82-year-old woman patient diagnosed with a familial form of amyotrophic lateral sclerosis (ALS). These patient-specific iPS cells with properties of ESCs have been successfully directed to differentiate into motor neurons, with the cell type destroyed in ALS (Dimos et al., 2008). In addition, skin fibroblasts-derived iPS cells can be successfully differentiated into insulin-producing isletlike clusters (ILCs) under feeder-free and serum-free conditions. The iPS-derived ILCs not only contain C-peptide positive and glucagon positive cells but also release C-peptide upon glucose stimulation. Thus, it is possible that patient-specific iPS cells can potentially provide a treatment for diabetes in the future (Tateishi et al., 2008).

Pluripotency can be induced in the differentiated murine and human cells by retroviral transduction of *Oct4*, *Sox2*, *Klf4*, and *c-Myc*. However, little is known about the molecular and cellular mechanism accompanying nuclear reprogramming, but transgene silencing is a prerequisite for normal cell differentiation. Subsequently, alkaline phosphatase (AP) is activated first and followed by stage-specific embryonic antigen 1 (SSEA1). Reactivation of endogenous *Nanog*, *Oct4*, *Sox2*, telomerase, and the silent X chromosome, marking fully reprogrammed cells, was observed in the late reprogramming process (Brambrink et al., 2008; Stadtfeld et al., 2008a).

5.1 Function of transcription factors

5.1.1 Oct4

Oct4, also called *Oct3/4* or *Pou5f1*, is one of the earliest transcription factors expressed in oocytes, ESCs, early embryos, primary germ cells, and adult stem cells (Okamoto et al., 1990; Rosner et al., 1990; Pesce et al., 1998; Pochampally et al., 2004), and orthologs was found in many animal species. The nuclear protein is encoded by a homeobox-containing gene named *Pou5f1*, which belongs to the family of POU (Pit Oct Unc) genes. It regulates gene transcription by binding DNA to octamer motifs ATGCAAT. Moreover, it plays a critical role in maintaining pluripotency and self-renewal of ESCs. Consistent with maintaining pluripotency, the expression of *Oct4* is rapidly downregulated during the formation of the trophoblast lineage. If the levels of *Oct4* are forcibly reduced by genetic manipulation, differentiation to trophectoderm will occur, whereas if the levels are raised, differentiation to endoderm will be promoted. Furthermore, the latest studies by Sonia and Michel demonstrate a gene dosage effect of *Oct4*, a factor at the cross-road between cell self-renewal and cell lineage specification (Sonia and Michel, 2007). Since *Oct4* expression is under a tight control of a smad pathway and a two- to eightfold increase in its expression is required to commit ESCs toward a cardiac lineage. The same phenomenon occurs *in vivo* in blastocysts to commit the epiblast toward the mesoderm (Zeineddine et al., 2006).

Oct4 is known to interact with other transcription factors to activate and repress gene expression in ESCs (Boyer et al., 2005). Genome scale location analysis (chromatin

immunoprecipitation coupled with DNA microarrays) of human ESCs reveals that *Oct4*, *Sox2*, and *Nanog* co-occupy the promoters of a large population of genes, and that many of these target genes encode the developmentally important homeodomain transcription factors (Pasquinelli et al., 2005). Therefore, Oct4, Sox2, and Nanog promote pluripotency and self-renewal of human ESCs through positive regulation of their target genes and the gene-encoding components of ESCs signaling pathways to form regulatory circuitry consisting of autoregulatory and feed-forward loops. In addition, a class of small noncoding RNAs known as micro-RNAs (miRNA) play vital roles in gene regulation and recent studies indicate that among the 14 miRNAs targeted by *Oct4*, *Sox2*, and *Nanog*, 3 (*mir-10a*, *mi-196b*, and *mir-448*) are specific to *Oct4* (Lagarkova et al., 2006). Consequently, miRNA genes are likely regulated by Oct4, Sox2, and Nanog in human ESCs and are important components of the transcriptional regulatory circuitry (Pasquinelli et al., 2005). For these reasons, it is not surprising that Nanog transduction is not necessary to induce pluripotency in the context of direct reprogramming because *Oct4* and *Sox2* expression may be sufficient to promote their endogenous activation. In support of this point, Nanog is reactivated in iPS clones at levels similar to ESCs (Blelloch et al., 2007; Meissner et al., 2007; Wernig et al., 2007).

5.1.2 Sox2

Sox2 (SRY-type high mobility group box 2) is a member of the *SOX* gene family that shares a similar HMG box DNA-binding motif (A/TA/TCAAA/TG) (Harley et al., 1994). So far, Sox2 is the only Sox-protein that has a crucial function in maintenance of ESCs pluripotency. In embryogenesis, the expression of *Sox2* shows a variety of dynamic expression patterns. It is expressed in the mature oocytes, ICM, epiblast of gastrulation, enteral mesoderm, branchial arch, and primary germ cells (Wood and Episkopou, 1999). Furthermore, unlike *Oct3*/*4*, *Sox2* is also expressed in the multipotential cells of the extraembryonic ectoderm. Reduction of *Sox2* expression in human ESCs results in loss of the undifferentiated stem cell state, as indicated by a change in cell morphology, altered stem cell marker expression, and increased expression of trophectoderm markers (Fong et al., 2008). In addition, in the process of generation of iPS cells, if *Sox2* is absent, the embryo stem cell-like clones can be formed, but they do not exhibit pluripotency (Takahashi et al., 2007b).

Therefore, *Sox2* and *Oct4* are both essential for the maintenance of ESCs pluripotency. They coregulate some gene transcription in embryogenesis, including *Fgf4, opn*, *Utf-1*, and *Fbx15* (Darr and Benvenisty, 2006). As mentioned above, *Sox2* regulatory elements in gene promoter regions are often found in close proximity to *Oct4* and *Nanog* binding sites. Sox2 can act synergistically with Oct4 *in vitro* to activate *Oct-Sox* enhancers, which regulate the expression of pluripotent stem cell-specific genes, including *Nanog, Oct4*, and *Sox2* themselves. Moreover, they cooccupy several hundred genes, often at apparently overlapping genomic sites in both mouse and human ESCs (Adhikary and Eilers, 2005).

5.1.3 c-Myc

The Myc-family genes belong to the basic-region/helix-loop-helix/leucine zipper (bHLHZip) transcription factor family and are associated with a number of cellular functions including cell growth, differentiation, proliferation, apoptosis, and tumorigenesis. c-Myc plays a central role in G1/S transition as an upstream regulator of cell cycle regulatory molecules because it can both activate and repress transcription of its target genes (Cartwright et al., 2005). In addition, exogenous c-Myc can promote self-renewal in ESCs, and sustained c-Myc activity can maintain ESCs self-renewal in the absence of leukemia inhibitory factor (LIF) (Cawley et al., 2004). Within the mammalian genome, there may be tens of thousands of Myc binding sites (Knoepfler et al., 2006), and Myc can influence global chromatin structure (Dominguez-Sola et al., 2007). In addition, c-Myc can interact with the prereplicative complex and promote DNA synthesis independent of transcriptional regulation (Jaenisch and Young, 2008).

Consequently, the role of c-Myc in inducing iPS cells may directly activate genes important for pluripotency or self-renewal and block differentiation through a widespread impact on chromatin by c-Myc to push differentiated cells to enter the cell cycle, which can contribute to iPS cell formation. When c-Myc expressed at high levels, the Myc protein could occupy a large number of genes and stimulate chromatin modifications by recruiting histone acetylase complexes that may provide increased access for transcription factors. Alternatively, DNA replication, promoted by Myc overexpression, can provide an opportunity for the somatic genome to reset its epigenetic state in the presence of exogenous reprogramming factors (Jaenisch and Young, 2002). In addition, c-Myc may also counter antiproliferative activation of p21 by Klf4 in iPS cells (Rowland et al., 2005).

5.1.4 Klf4

Klf4 belongs to Krüppel-like factors (KLFs), acted as a transcriptional activator or repressor, and can regulate genes involving in proliferation and differentiation, which function as a tumor suppressor or oncogene depending on the functional status of $p21^{Cip1/WAF1}$, a cyclin-dependent kinase inhibitor. In *p21* null cells, Klf4 can promote cell proliferation by downregulating *p53*, a negative regulator of *Nanog* during ESCs differentiation (Li et al., 2005). On

the other hand, *Klf4* is, like *c-Myc*, a downstream target of activated STAT3 in LIF-induced ESCs. Overexpressed Klf4 in mouse ESCs can promote self-renewal and Klf4-transduced embryoid body (EB) can lead to sustained expression of *Oct4* and inhibition of differentiation (Nakatake et al., 2006). In addition, *Klf4* cooperated with *Oct4* and *Sox2* to activate the *Lefty1* core promoter in mouse ESCs (Jiang et al., 2008). Simultaneously, the depletion of *Klf2*, *Klf4*, and *Klf5* leads to ESCs differentiation and Klf proteins share many targets of *Nanog* revealing that core Klf circuitry can be integrated into the *Nanog* transcriptional network to specify gene expression that is unique to ESCs (Alon, 2007). Klf4's function in reprogramming somatic cells to iPS cells may involve activation of specific ESCs genes acting as a cofactor of *Oct4* and *Sox2* and counterweight for proapoptotic properties of c-Myc through p53 repression (Lin et al., 2005).

Taken together, the four transcription's function in generation of iPS cells may be as follows: Myc proteins probably loosen the chromatin structure of somatic cells by binding to numerous sites throughout the genome and by recruiting multiple histone acetylase complexes, which enable Oct4 and Sox2 to bind to their target genes. When Oct4 and Sox2 overexpressed exogenously, they may contribute directly to the activation of endogenous *Oct4*, *Sox2*, and *Nanog*, the products of which in turn contribute to the maintenance of their own gene expression and pluripotentcy (Alon, 2007). Klf4 may also act as a cofactor of Oct4 and Sox2 to activate specific ESCs genes (Jiang et al., 2008). The balance of four transcription factors contributes to generation of iPS cells but does not transform cells, or tumor cells, or apoptosis.

5.2 Potential applications and problems of iPS cells

About 128 million persons in the world suffer from chronic and degenerative diseases, and cell transplantation therapies based on stem cells hold great promise in the treatment of these diseases. However, the shortage of donor cells severely limits the process of cell replacement therapies. Now, generation of human iPS cells can hopefully provide an easily accessible and cost-effective method to create patient-specific PSCs, which will usher in an era of regenerative medicine. For transplantation therapies, with the exception of autoimmune diseases, patient-specific iPS cells should largely eliminate the immune rejection. In addition, Human iPS cells would be useful for studying the development and function of human tissues, for understanding disease mechanisms in the production of new disease models, and for discovering and screening the specific drugs to cure the diseases, which would greatly speed up the process of these drugs to market. Moreover, iPS cells, generated from somatic cells can induce nearly complete reprogramming. Consequently, they will help to study and discuss the mechanism of reprogramming.

It is important to understand, however, that before the iPS cells can be used in the clinic, additional work is required to avoid integrating vectors into the genome, potentially introducing mutations at the insertion site. In the process of generation of iPS cells, exogenous genes are randomly inserted into the donor cells genome; thus, it may affect some certain important gene expression at the risk of insertional mutagenesis. Even more seriously, the transfection of donor cells needs virus as vector, which is unsafe for cell therapy. Therefore, currently, it is necessary to seek for a new method to induce somatic cell reprogramming without virus. An alternative would be the use of small molecules to penetrate cell walls and turn on production of the necessary transcription factors. On the other hand, the continued presence of transgenes encoding oncogenic factors, such as c-Myc, delivered by oncogenic retroviruses can turn on cancer-causing genes. Studies showed that approximately one-fifth of the only live F1 mice from germline iPS chimeras developed tumors, which may be due to the reactivation of c-Myc retrovirus (Okita et al., 2007). Nevertheless, the generation of iPS cells may also be induced from mouse and human fibroblasts without c-Myc. In addition, cell transplantation therapy should be nonpathogenic, but in the culture of hESCs usually animal serum, the antibody and matrix protein are used and most of them are animal-derived products. There is a potential possibility of contaminated animal virus, including those not yet known. However, studies reported autogenic feeders that differentiated from the same ESCs or human feeder cells that are prescreened for pathogens could be used to culture hESCs. Also, without feeder cells, ESCs can grow with the appropriate incitement signals (Klimanskaya et al., 2008). Interestingly, in the process of inducing iPS cells, the use of serum-free medium appeared not only to be selected for reprogrammed cells but also to accelerate reprogramming (Blelloch et al., 2007).

6 Conclusions

This review summarizes the currently available methods to generate PSCs from somatic cells. Each method has advantages as well as disadvantages over other methods in the aspect of potential therapies. IPS cells, generated from somatic cells by virus-mediated transfection with transcription factors can induce nearly complete reprogramming and share many properties with ESCs, such as in morphology, proliferation, and pluripotency. The generation of iPS cells can hopefully provide an easily accessible and cost-effective method to create patient- and disease-specific PSCs. They are invaluable for disease and drug research and, potentially, would usher in an era of regenerative medicine. Moreover, the process of generation of iPS cells is useful to study the mechanism of reprogramming. However, before the iPS cells can be used in the clinic, additional work is required to avoid

integrating vectors into the donor cells genome and using oncogene as inducing factor. An alternative would be the use of small molecules to penetrate cell walls and turn on production of the necessary transcription factors. Moreover, it will be critical for any application of reprogrammed somatic cells in a medical setting to bypass the stable overexpression of retroviral transgenes by using a transient expression system.

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