Review Article

Advances on the study of induced pluripotent stem cells

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ABSTRACT Recently, great breakthroughs of induced pluripotent stem (iPS) cells have been made since the landmark work by Yamanaka and colleagues in 2006. It opened up a new era in the realm of somatic cell reprogramming and updated our traditional concepts about pluripotency control. Nuclear reprogramming is of great medical interest, as it has the potential to generate a source of patient-specific cells. iPS cells technology brings a new appreciation for the possibility of reprogramming differentiated cells. With the iPS cell as the functional equivalent of the ES cell, any cell could then be converted to any other cell types. Here, we'll review the strategies of reprogramming somatic cells to a pluripotent embryonic state and mainly discuss the development of iPS cells, as well as its application in animal models, and present the recent insights into the problems regarding the iPS cells safety.

KeyWords: iPS cells, embryonic stem (ES) cells, reprogramming, pluripotency, epigenetic modification

In normal development, cells transit in a unidirectional process from the totipotent zygote to pluripotent inner cell mass (ICM) and epiblast cells and to more restricted and eventually differentiated cells. These transitions occur in the context of the embryo as a result of cell-cell interactions and are characterized by distinct epigenetic modifications and remarkable accuracy [1, 2]. It is long thought that the differentiation process is irreversible until the advent of cloning technique [3]. Embryonic stem (ES) cells are derived from the ICM of mammalian blastocysts, have the ability to grow indefinitely while maintaining pluripotency and can differentiate into cells of all three germ layers [4, 5]. Previous studies showed ES cells might be used to treat a lot of diseases, such as Parkinson's disease, spinal cord injury, and diabetes [6]. However, there are ethical problems about the use of embryos, as well as tissue rejection after transplantation in patients. One way to resolve these issues is the generation of pluripotent stem cells directly from the patients' own cells.

In 1996, Wilmut and colleagues made breakthrough in reproductive cloning. They generated an exact replica of a higher mammal, the Dolly by transferring a nucleus from a fully differentiated cell in the mammary gland [7]. This work demonstrated that the genome of differentiated cells can be reprogrammed into an embryonic state and then to resume the developmental process to generate a normal adult, which encouraged a new research direction called "therapeutic cloning" later [8], or referred to as somatic cell nuclear transfer (SCNT). Other somatic cell reprogramming strategies include fusion of somatic cells with ES cells [9-12] or treatment with extracts of pluripotent cells [13, 14], culture-induced reprogramming [15, 16] and so on. Yet all have problems in practical application due to technical issues.

A promising alternative to SCNT was proposed by Yamanaka and colleagues in 2006 [17], describing that the reprogramming process can be achieved with the retroviral-mediated introduction of only four transcription factors into fibroblasts. The expression of four transcription factors, Oct4/Sox2/cMyc/Klf4, was able to convert somatic cells from a terminally differentiated state to an embryonic level, these cells were designated iPS (induced pluripotent stem) cells. Since then, this work was well received with great excitement and made breathtaking progresses [18]. Here, we will have a review of the iPS cells advances. In the article, we will start with an overview of somatic cell reprogramming strategies and the development of iPS cell establishment, discuss the key steps in generating iPS cells and several relevant scientific issues, and evaluate its current restrictions and promises in future research.

1. Overviews of Strategies for Reprogramming Somatic Cells

Several different strategies such as nuclear transplantation (NT), cellular fusion, and culture induced reprogramming have been employed to induce the conversion of differentiated cells into an embryonic state (Fig.1). These experimental approaches have been extensively reviewed [19-21] and will only be briefly summarized here.

The authors have no commercial, proprietary, or financial interest in the products or companies described in this article.

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Submitted January 12, 2009. Accepted for publication March 30, 2009.
Available at www.lymphologyandoncology.com

ISSN: 1538-5124/$ - see front matter

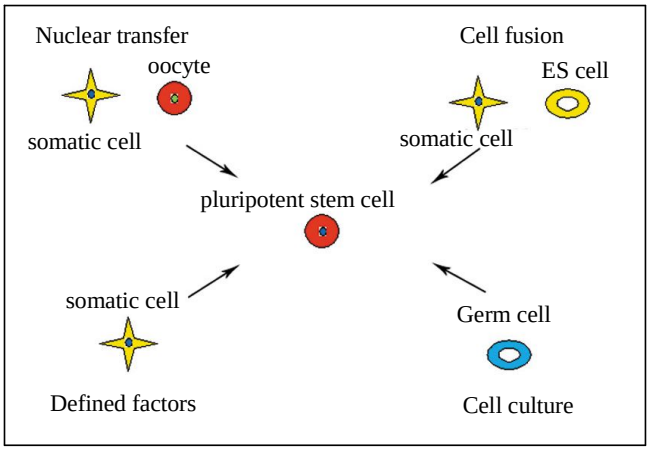

Fig. 1 Strategies to induce epigenetic reprogramming

Currently, four strategies are used to induce reprogramming of somatic cells, including nuclear transfer, cell fusion, cell explantation in culture, and transduction with defined factors, which are illustrated in the figure.

1.1 Nuclear Transplantation

Nuclear transfer provided evidence for the notion that irre versible alterations of the genome are not required for normal de velopment. Successful nuclear transfer was first reported in 1952, showing that nuclei from blastulastage embryos into enucleated Rana pipiens eggs resulted in normal hatched tadpoles [22]. Then a breakthrough came in 1996, when Wilmut and colleagues pro duced an adult sheep, famously known as "Dolly," using nuclei de rived from follicle cells [7]. However, Cloning from terminally differentiated donor cells is inefficient and in many cases successful only when a "two-step" procedure was used: ES cells were derived from cloned embryos and animals were then made from those ES cells, indicating that the differentiation state of the donor cell affects the efficiency of producing cloned animals, with less differentiated cells being more subject to epigenetic reprogramming. Moreover, a significant issue when considering the potential of nuclear transfer strategies for generating patient-specific human ES cell lines is the availability of human oocytes. However, Egli et al have found that it is possible to generate pluripotent cells by nuclear transfer using adult somatic cells as donors and zygotes as recipients [23]. This strategy adaptation would solve major practical problems that prevent the application of NT for medicine.

1.2 Reprogramming by Fusion with ES Cells

Fusion between different cell types has been used to study the plasticity of the differentiated state [24]. Epigenetic reprogramming of somatic nuclei to an undifferentiated state has been demonstrated in murine hybrids produced by fusion of embryonic cells with somatic cells. Hybrids between various somatic cells and embryonic carcinoma cells [25], embryonic germ (EG), or ES cells [26] share many features with the parental embryonic cells, indicating that the pluripotent phenotype is dominant in such fusion products. However, the inefficiency of the fusion process has impeded the study of molecular mechanisms involved in somatic reprogramming. Moreover, whether somatic genomes are fully reprogrammed by fusion remains to be resolved. In thymocyte-ES hybrid cells, the promoter regions of several genes, including Oct-3/4, in the thymocyte genome acquired ES-like epigenetic status, including histone acetylation and methylation [27]. Therefore, at least a part of the somatic genome is reprogrammed by fusion. Rejection upon implantation remains an issue with hybrid cell because of the ES cell-derived chromosomes.

1.3 Culture–Induced Reprogramming

Under certain physiological conditions, entire cells can de-differentiate or transdifferentiate into another cell fate. Pluripotent cells have been derived from embryonic sources such as blastomeres and the inner cell mass (ICM) of the blastocyst (ES cells), primordial germ cells (EG cells), and postnatal spermatogonial stem cells [28, 29]. Verfaillie and associates reported the development of pluripotent stem cells after the prolonged culture of bone marrow-derived cells [30]. Shinohara and associates demonstrated that pluripotent stem cells can be generated during the course of culture of germline stem (GS) cells from neonate mouse testes, which were termed as multipotent germline stem (mGS) cells [31]. Reprogramming from spermatogonial stem cells cannot apply to females. As an alternative, however, histocompatible ES cells can also be generated by parthenogenesis.

1.4 Reprogramming by Defined Transcription Factors

In 2006, Takahashi and Yamanaka [17] surprised the world by reporting that retrovirus-mediated introduction of four transcription factors (Oct-3/4, Sox2, c-Myc, and KLF4) into mouse embryonic or adult fibroblasts and selection for the expression of Fbx15, a target of Oct-3/4 and Sox2, resulted in the generation of induced pluripotent stem (iPS) cells, which are similar to ES cells in morphology, proliferation, and teratoma formation. Fbx15-selected iPS cells are, however, significantly different from ES cells in gene expression and DNA methylation patterns. When transplanted into blastocysts, iPS cells only give rise to chimeric embryos, but not adult or germline competent chimeras. These data indicate that reprogramming in Fbx15-selected iPS cells is incomplete.

Afterwards, three groups confirmed the idea and made significant improvements in the generation of iPS cells [32-34]. They generated iPS cells competent for adult and germline chimeras by using a more stringent selection marker, Nanog. Although both Fbx15 and Nanog are targets of Oct-3/4 and Sox2, the former is dispensable for pluripotency, while the latter plays crucial roles. Nanog-selected iPS cells are almost indistinguishable from ES cells in global gene expression [33], DNA methylation, and histone modification [32, 34]. Oct-3/4 can also be used as a stringent selec

tion marker for iPS cell induction [34]. These data demonstrated that full reprogramming can be achieved by expression of the four factors and using an appropriate selection procedure. Later, another three papers reported the isolation of reprogrammed pluripotent cells from genetically unmodified somatic cells based on morphological criteria instead of any reporter selection, which is of potential therapeutic value [35-37].

Shortly after that, human iPS cells were reported by three independent groups, surprisingly employing different combination of factors including Oct4, Sox2, Myc, Klf4, Nanog, Lin28, SV40L-T, and HTERT [38-40]. Then successful treatment of mouse sickle cell anemia by iPS cells derived from autologous skin was reported, paving the way for the application of iPS technology in disease therapies [41]. Very recently, the production of iPS cells can be achieved without viral transduction [42], leading the magic continues for the iPS cells strategy.

2. Defined factors directly reprogram somatic cells in mouse

2.1 Fbx15–iPS cells by defined factors

Somatic cells can be reprogrammed by transferring their nuclear contents into oocytes [7] or by fusion with ES cells [9, 11], indicating that unfertilized eggs and ES cells contain factors that can confer totipotency or pluripotency to somatic cells. It seemed that the factors that play important roles in the maintenance of ES cell identity also play vital roles in the induction of pluripotency in somatic cells. Based on this hypothesis, Takahashi and Yamanaka in 2006 tested 24 different candidate factors for their ability to induce pluripotency and achieved a significant breakthrough in reprogramming somatic cells back to an ES-like state [17].

They successfully reprogrammed mouse embryonic fibroblasts (MEFs) and adult fibroblasts to pluripotent ES-like cells after viral-mediated transduction of the four transcription factors Oct4, Sox2, c-Myc, and Klf4 followed by selection for activation of the Oct4 target gene Fbx15 [43]. Those cells that had activated Fbx15 were termed induced pluripotent stem (iPS) cells and were shown to be pluripotent by their ability to form teratomas. However, they are different from ES cells with regards to gene expression and DNA methylation patterns, and fail to produce adult chimaeras. The pluripotent state was dependent on the continuous viral expression of the transduced Oct4 and Sox2 genes, whereas the endogenous Oct4 and Nanog genes were not expressed or expressed at a lower level than in ES cells. This is consistent with the conclusion that the Fbx15-iPS cells only represented an incomplete state of reprogramming.

2.2 Nanog/Oct–4–iPS cells by defined factors

In 2007, three groups used the activation of the endogenous Oct4 or Nanog genes as a more stringent selection criterion for pluripotency, and generated the Nanog-iPS or Oct4-iPS cells [32-34]. In contrast to Fbx15-iPS cells, the pluripotent state in Oct4 or Nanog-iPS cells depended on the activity of the fully reprogrammed and hypomethylated endogenous Oct4 and Nanog promoters but not on the viral transduction factors. They found that global gene expression and the chromatin status of Oct4 or Nanog selected iPS cells were indistinguishable from those of ES cells, and the inactive X chromosome of the somatic donor cells was reactivated in iPS cells [32]. Most Importantly, Oct4 and Nanog-iPS cells generated postnatal chimeras, contributed to the germ line [32-34], and generated late gestation embryos through tetraploid complementation [34], which is the most stringent test for developmental potency. Thus, all molecular and biological evidence indicated that Oct4 and Nanog-iPS cells were indistinguishable from ES cells.

2.3 Drug–free iPS cells

The original isolation of iPS cells was based upon retrovirus-mediated transduction of oncogenes and on drug-dependent selection for Fbx15, Oct4, or Nanog activation. These two aspects seriously hinder the application of the in vitro reprogramming approach for therapeutic use in humans because mice derived from iPS cells frequently developed cancer [33, 44] and because the isolation of human iPS cells cannot be based on genetically modified donor cells. To circumvent this problem, three groups attempted to generate iPS clones without drug selection and succeeded in obtaining iPS cell lines indistinguishable from G418 selected clones [35-37]. Fully reprogrammed, genetically unmodified mouse fibroblasts were isolated based only on morphological criteria, as reprogramming occurred frequently enough to be detectable in culture. Subsequent to these studies, human iPS cells were isolated from genetically unmodified fibroblasts [38-40], which brought this technology one step closer towards patient-specific pluripotent cells for cell replacement therapy.

2.4 Questions about iPS cells induction

Which of the original factors are essential for the reprogramming process? Genetic experiments had established that Oct4 and Sox2 are essential for pluripotency [45-47]. It is possible that Oct4, which in normal development is already expressed in the oocyte and may be the most upstream gene in the molecular circuitry of pluripotency, is the only obligatory factor to initiate reprogramming and that other factors serve to accelerate the process and to increase efficiency. Nonetheless, the exact role of the two oncogenes c-Myc and Klf4 in reprogramming is less clear. It appears that c-Myc significantly enhances and accelerates the process but is dispensable for reprogramming, as both mouse and human iPS cells have been obtained in the absence of c-Myc transduction [38, 44, 48].

After the first mouse ESC lines were derived in 1981 [4, 5] many efforts were made to establish rat ESCs, without success to

date [49-55]. Recently, researchers report the generation of induced pluripotent stem cells (iPS cells) from adult rat cells and demonstrate that the iPS cells technique provides a feasible approach to establish pluripotent stem cells for a species in which ESCs have previously proven to be difficult to establish from the early embryo [56].

3. Human iPS cells

In 2007, the Yamanaka group at Kyoto University and Thomson group in Wisconsin published independently the successful generation of human iPS cells from various somatic cells [38, 39]. The milestone studies by the two groups shocked the scientists all over the world and pushed iPS cells research to the hotspot of stem cell science. The Yamanaka group demonstrated the generation of iPS cells from adult human dermal fibroblasts with the same four factors: Oct3/4, Sox2, c-Myc, and Klf4 that worked for mouse cells. Human iPS cells were similar to human embryonic stem (ES) cells in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity. Furthermore, these cells could differentiate into cell types of the three germ layers in vitro and in teratomas [39].

However, Yu from the Thomson group identified 4 factors out of 14 candidates to be sufficient for the induction of iPS cells from ES-derived CD45+ hematopoietic cells [38]. They showed that four factors Oct4, Sox2, Nanog, and Lin28 are sufficient to reprogram human somatic cells to pluripotent stem cells that exhibit the essential characteristics of embryonic stem (ES) cells. These induced pluripotent human stem cells have normal karyotypes, possess telomerase activity, express cell surface markers and genes that characterize human ES cells, and maintain the developmental potential to differentiate into advanced derivatives of all three primary germ layers. Such induced pluripotent human cell lines should be useful in the production of new disease models and in drug development, as well as for applications in transplantation medicine.

In fact, the two independent studies suggest that there could be more factors potentially important for the reprogramming process. Subsequently, Daley and colleagues reported that hTERT and SV40 large T can enhance the reprogramming efficiency of Oct4/Sox2/Myc/Klf4 on human somatic cells [40]. These data demonstrate that defined factors can reprogramme human cells to pluripotency, and establish a method whereby patient-specific cells might be established in culture.

4. The promise of iPS cells for therapy

There are several hurdles to be overcome before iPS cells can be considered as a potential patient-specific cell therapy, and it will be crucial to characterize the developmental potential of human iPS cell lines [57] (Fig.2). As a research tool, iPS cells technology provides opportunities to study normal development and to understand reprogramming. iPS cells can have an immediate impact upon models for human diseases, including cancer. In 2007, Jaenisch group in America reported the first exciting result of iPS cells therapy in mice [41]. They used a humanized knockin mouse model for sickle cell anemia, and generated iPS cells from these knock-in mice. The harmful allele was subsequently replaced by β A allele in iPS cells through homologous recombination. Then, the corrected iPS cells were induced to differentiate into hematopoietic progenitors and transplanted back to the sick mice. These progenitors derived from β A corrected iPS cells were able to contribute 70% of the peripheral blood and mice were effectively cured of sickle cell anemia.

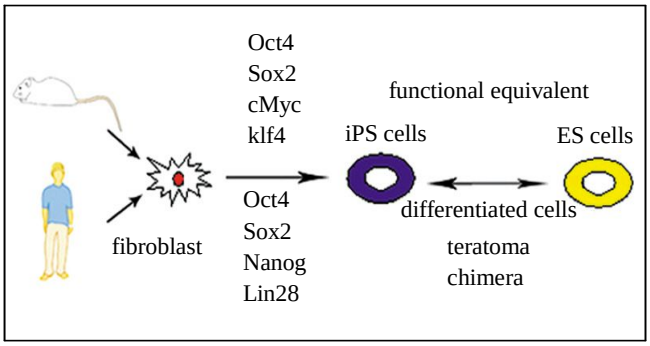

Fig. 2 Schematic of direct reprogramming

Fibroblasts from mouse or human are transduced with Oct4, Sox2, c-Myc, and Klf4 or Oct4, Sox2, Nanog, and Lin28, and can be reprogrammed into induced pluripotent stem (iPS) cells, which serve as functional equivalent of ES cells in terms of differentiation potential, teratoma formation, and chimera derivation.

Amazingly, another work by Jaenisch and colleagues explored the therapeutic potential of iPS cells for neural cell replacement strategies [58]. They showed that iPS cells can be efficiently differentiated into neural precursor cells, giving rise to neuronal and glial cell types in culture. Upon transplantation into the fetal mouse brain, the cells migrate into various brain regions and differentiate into glia and neurons, including glutamatergic and GABAergic subtypes. Electrophysiological recordings and morphological analysis demonstrated that the grafted neurons had mature neuronal activity and were functionally integrated in the host brain. Furthermore, iPS cells were induced to differentiate into dopamine neurons of midbrain character and were able to improve behavior in a rat model of Parkinson's disease upon transplantation into the adult animal brain.

In addition, two groups recently reported the first iPS cell lines derived from patients with various genetic diseases and the generation of iPS cells-derived differentiated cells [59, 60]. With the development of viral-free induction technique [42], which will be discussed later in this article, the generation of iPS cells will be much safer. Recently, Jaenisch and colleagues show that fibroblasts from patients with idiopathic Parkinson's disease can be efficiently reprogrammed and subsequently differentiated into dopaminergic neurons. Moreover, they derived iPS cells free of reprogramming

factors using Cre-recombinase excisable viruses. Factor-free hiPS cells maintain a pluripotent state and show a global gene expression profile, more closely related to hESCs [61].

The long term goal of reprogramming approaches is to create patient-specific donor cells for transplantation therapy, avoiding immunorejection, a major complication in current transplantation medicine. iPS cells derived from somatic cells of patients represent a powerful tool for biomedical research and may provide a source for replacement therapies [57]. However, only the safety problems regarding viruses encoding the reprogramming factors resolved can the technique be applied to the patients in the near future.

5. Roles of reprogramming factors

5.1 Oct–3/4

Oct-3/4 was identified as an Oct family protein, containing the POU domain, specifically expressed in embryonic carcinoma (EC) cells, early embryos, and germ cells [62-64]. The transcription factor Oct3/4 is essential for the maintenance of self-renewal of pluripotent cells. Oct-3/4 null embryos die in utero during the peri-implantation stages of development [65]. ES cells cannot be derived from Oct-3/4 null blastocysts. Oct-3/4 also plays important roles in promoting differentiation and its expression level is an important determinant of the cell fate in mouse ES cells. Studies revealed that Oct4, Sox2, and Nanog bind together at their own promoters to form an interconnected autoregulatory loop. Genome-wide studies revealed a large panel of target genes with Oct-regulatory elements, most of which possess regulatory elements for the transcription factors Sox2 and Nanog in close proximity that were found to be co-occupied in genes specifically positively or negatively regulated in ES cells [66, 67].

5.2 Sox2

The transcription factor Sox2 is part of a large protein family that shares a similar high mobility group (HMG) box DNA-binding motif. Like Oct-3/4, Sox2 also marks the pluripotent lineage of the early mouse embryo; it is expressed in the ICM, epiblast, and germ cells. Unlike Oct-3/4, however, Sox2 expression is also associated with uncommitted dividing stem and precursor cells of the developing central nervous system [68, 69]. Sox2, like Oct-3/4, is essential for the maintenance of pluripotency. Sox2 null embryos die at the time of implantation due to a failure of epiblast development [70]. Sox2 regulatory elements in gene promoter regions are often found in close proximity to Oct3/4 and Nanog binding sites [66], as mentioned in the Oct3/4 section.

5.3 Nanog

Traditional biochemical and genetic approaches have revealed that Oct4, Sox2, and Nanog together are required for the maintenance of ES cell pluripotency and selfrenewal [71, 72] and are central to the transcriptional regulatory network that governs pluripotency. Nanog contains divergent homeodomain protein, and is expressed in Morula, ICM, epiblast, ES cells, and germ cells. It is an important regulator of ES cell pluripotency and germ cell development; deletion of Nanog in the mouse results in embryonic lethality (E5.5) and Nanog null ES cells lose pluripotency. It can be replaced by c-Myc and Klf4 in human study and not required for reprogramming in either human or mouse [17, 39].

5.4 c–Myc

c-Myc is a multidomain transcription factor and potent oncogene extensively implicated in many aspects of cellular biology, including cell proliferation, DNA replication, inhibition of cellular differentiation, and cell growth [73-77]. It has a multitude of target sites throughout the genome [78] and plays a role in regulation of protein coding [73, 79] and noncoding microRNA genes [80, 81]. The N terminus of Myc protein binds to several proteins including TRRAP [82], while its C terminus contains the basic region/helixloop-helix/leucine zipper (BR/HLH/LZ) domain, through which Myc binds to a partner protein Max. The Myc-Max dimers bind to a DNA sequence CACA/GTG, which is a subset of the general E box sequence CANNTG that is bound by all bHLH proteins. c-Myc has been regarded as a major downstream target for two pathways that support maintenance of pluripotency: the LIF (leukaemia inhibitory factor)/STAT3 and the Wnt signalling cascades. ES cells, expressing a stable c-Myc mutant, could contribute to chimeric mice even when cultured in absence of LIF [83], demonstrating the potential of c-Myc in stem cell renewal.

5.5 Klf4

Klf4 belongs Krüppel-like factors (KLFs), which are zinc-finger proteins that contain amino acid sequences resembling those of the Drosophila embryonic pattern regulator Krüppel [84]. Klf4 is, like c-Myc, a downstream target of activated STAT3 in LIF-induced ES cells. Its overexpression leads to sustained expression of Oct3/4 and inhibition of differentiation in ES cells [85]. Similar to Sox2, Klf4 can also act as a co-factor for Oct3/4-mediated regulation of gene transcription. Klf4 can function both as a tumor suppressor and an oncogene [86]. Klf4 null embryos develop normally, but newborn mice die within 15 hours and show an impaired differentiation in the skin [87] and in the colon [88], thus indicating that it plays a crucial role as a switch from proliferation to differentiation.

5.6 Lin28

Lin28 was expressed in oocyte, zygote, blastocyst and to varying degrees during organogenesis; and in adult, expressed in the bone, liver, and gonads. It has several RNA-binding domains, playing a role in regulating mRNA translation or stability. Lin28 was

transferred from the nucleus to the cytoplasm for processing P-bodies. The P-bodies are sites of mRNA degradation and microRNA regulation [89]. Its homologs in Drosophila, Xenopus, and mouse appear to be expressed and down-regulated during development, which is consistent with a conserved role for the factor as a regulator of developmental timing [90]. Thus, Lin28 could act to stabilize newly synthesized transcripts to allow for adoption of an ES cell transcriptional network in the context of reprogramming.

6. Perspectives regarding the iPS cells future

6.1 ES cells or iPS cells

iPS cells technology is a novel method for generating pluripotent stem cells [20, 21, 91]. The method is striking and especially attractive for researchers in that it can convert somatic cells directly into pluripotent cells in a manner that is totally independent of the availability of embryonic cells. It is different from other techniques for generating pluripotent stem cells, for instance, the derivation of ES cells from embryos generated by NT [19]. Opponents of stem cell research have welcomed iPS cells technology for achieving an embryonic-like state without the ethical dilemma of destroying human embryos. Media reports of iPS cell advances also have enlightened the public's hopes of producing human iPS cells to generate patient-specific stem cells for disease research, drug development, and new cell-based therapies [92].

Some scientists, therefore, might be tempted to jump to the conclusion that research on human embryonic stem cells (hES cells) is unnecessary in light of the emerging possibility of human iPS cell research. However, it would be a serious mistake to consider that recent developments in iPS cells research can replace the need for hES cells research [92]. SCNT will remain the standard in the generation of cloned ES cells and reprogramming research, thus, hES cell may not be readily replaced by iPS cells [18]. We should maintain a realistic perspective on iPS cell research hand in hand with hES cell research. Both cell types can be used as the pluripotent source for differentiated cells or tissues in regenerative medicine [93, 94]. Moreover, the pressing scientific question, how closely do iPS cells resemble conventional ES cells, should be settled before this technique was applied to clinic [57]. Therapeutically, the iPS technology is still unsuitable for human diseases at present, due to safety concerns associated with viral transduction of those potentially oncogenic factors [41].

6.2 Reprogramming efficiency improvement

Reprogramming of mouse and human somatic cells can be achieved by ectopic expression of transcription factors, but the reprogramming process is efficient, which is related to the epigenetic status of the reprogrammed cell, especially the DNA methylation [17, 21]. Although the iPS cells selected by using Oct4 or Nanog were much closer to ES cells by all means used to characterize them, the efficiency of generating the stable iPS clones was still low (0.03% when using Nanog, and 0.08% when using Oct4) [33, 34]. Studies showed that adult mouse neural stem cells express higher endogenous le vels of Sox2 and c-Myc than embryonic stem cells, and that exogenous Oct4 together with either Klf4 or c-Myc is sufficient to generate iPS cells from neural stem cells [95]. It is proposed that, in inducing pluripotency, the number of reprogramming factors can be reduced when using somatic cells that endogenously express appropriate levels of complementing factors.

Douglas Melton reported that DNA methyltransferase and histone deacetylase (HDAC) inhibitors could improve reprogramming efficiency [96]. In particular, valproic acid (VPA), an HDAC inhibitor, improved reprogramming efficiency by more than 100-fold, using Oct4-GFP as a reporter. VPA also enabled efficient induction of pluripotent stem cells without introduction of the oncogene c-Myc. The recent work by Deng et al found that p53 siRNA and UTF1 enhanced the efficiency of iPS cells generation up to 100-fold, even when the oncogene c-Myc was removed from the combinations [97]. In the future, this is expected to facilitate the establishment of patient-specific and disease-specific human iPS cells.

6.3 Oncogenic of the iPS cells

There is a concern of safety as the four transcription factors Oct4/Sox2/Myc/Klf4 are oncogenic or potentially oncogenic. In Yamanaka's work, indeed, about 20% of the F1 mice developed cancer, probably due to the reactivation of c-Myc [17]. Later, iPS cells generation without introduction of the oncogene c-Myc was also accomplished by this team [44], showing very low occurrence of tumorigenicity. Other experiments showed that c-Myc is dispensable for reprogramming [38, 48]. In addition, retroviruse vectors themselves can cause insertional mutagenesis and activate endogenous oncogenes. Future efforts should be focused on identifying alternatives to the viral delivery system and reprogramming factors [98] (Fig.3).

Two transient transfection reprogramming methods have been published to address this issue [99, 100]. Very recently, studies showed that non-viral transfection of a single multiprotein expression vector, which comprises the coding sequences of c-Myc, Klf4, Oct4 and Sox2 linked with 2A peptides, can reprogram both mouse and human fibroblasts [42]. This system minimizes genome modification in iPS cells and enables complete elimination of exogenous reprogramming factors, efficiently providing iPS cells that are applicable to regenerative medicine, drug screening and the establishment of disease models. Since the transcriptional factors represent definable signaling pathways, it is rational, some day in the future, to propose a chemical approach to switch on these pathways for iPS reprogramming [18]. Anyway, these problems must be resolved before this new approach of iPS cells generation can be applied to human diseases therapeutics.

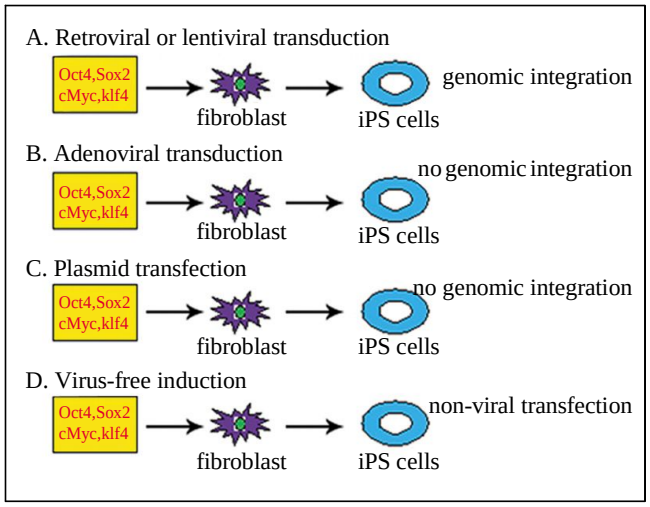

Fig. 3 Sketch of the strategies to generate iPS cells

Retroviral transduction approach initially used by Yamanaka et al possesses the potential of genomic integration, while strategies of adenoviral, plasmid transfection, and the recent virus-free induction avoid ectopic DNA insertion.

Acknowledgements

We are very grateful to Dr Xing Wu, Dr Hongying Sha, and all members of the stem cell laboratory for critical reading of the manuscript. This research was partially supported by the National Natural Science Foundation of China (30870805).

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