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Induced pluripotent stem cells, new tools for drug discovery and new hope for stem cell therapies

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Abstract

Somatic cell nuclear transfer or therapeutic cloning has provided great hope for stem cell-based therapies. However therapeutic cloning has been experiencing both ethical and technical difficulties. Recent breakthrough studies using a combination of four factors to reprogram human somatic cells into pluripotent stem cells without using embryos or eggs led to an important revolution in stem cell research. Comparative analysis of human induced pluripotent stem cells and human embryonic stem cells using assays for morphology, cell surface marker expression, gene expression profiling, epigenetic status, and differentiation potential revealed a remarkable degree of similarity between these two pluripotent stem cell types. This mini-review summarizes these ground-breaking studies. The advance in reprogramming will enable the creation of patient-specific stem cell lines to study various disease mechanisms. The created cellular models will provide valuable tools for drug discovery. Furthermore, this reprogramming system provides great potential to make customized patient-specific stem cell therapy with economical feasibility.

Keywords

reprogramming; pluripotency; embryonic stem (ES) cells; induced pluripotent stem (iPS) cells; stem cell therapy; regenerative medicine

Pluripotent stem cells have provided great hope for cell replacement therapies because of their ability to self-renew and their potential to form all cell lineages in the body [1]. Human embryos are the main sources for producing human pluripotent stem cells that are genetically unmodified so far. Alternative methods for producing pluripotent stem cells include somatic cell nuclear transfer or therapeutic cloning, which involves replacing the genetic material of unfertilized or newly fertilized eggs with that from an adult cell of patients and then forcing the cell to divide to create an early-stage embryo [2,3], and fusion of fibroblasts with embryonic stem (ES) cells [4,5]. However the therapeutic application of either approach has been experiencing both ethical and technical difficulties [6]. Reprogramming human somatic cells into induced pluripotent stem (iPS) cells without the need of embryos or eggs will solve the technical and ethical problems.

With the studies led by Yamanaka, Jaenisch, Hochedlinger, Thomson, and Daley, an important revolution in stem cell research is undertaken. Using a cocktail of four factors, somatic cells can be reprogrammed into induced pluripotent stem cells [7-13]. These advances will enable the creation of patient-specific stem cell lines for the study of various disease mechanisms and provide valuable tools for drug discovery. Once the safety issues are solved, this reprogramming system will make the production of customized patient-specific tissues from

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patients' own somatic cells for cell-replacement therapies more feasible [14]. A summary of progress in iPS cell induction is shown in Table 1.

Several transcription factors, including Oct4, Sox2, and Nanog, function in the maintenance of pluripotency in ES cells [15-19]. Other genes that are frequently upregulated in tumors, such as Stat3, c-Myc, Klf4, and β -catenin, have also been shown to contribute to the maintenance of ES cell phenotype and rapid proliferation of ES cells [20-25]. In the study by Takahashi and Yamanaka in 2006, 24 genes that have been shown to function in the maintenance of ES cell pluripotency or rapid proliferation were selected as candidates to induce pluripotency in somatic cells [7]. A β -geo cassette (a fusion of the β -galactosidase and neomycin resistance genes) was inserted by homologous recombination into mouse Fbx15, a gene that is specifically expressed in mouse ES cells, to select for reprogramming events that activate the Fbx15 locus. Transduction of all 24 candidates together into mouse embryonic fibroblasts generated clones that exhibited morphology similar to ES cells.

To determine which of the 24 candidates were critical, individual factors were withdrawn from the pool of the transduced candidate genes [7]. Ten factors were identified, whose individual withdrawal from the bulk transduction pool resulted in no colony formation 10 days after transduction and fewer colonies 16 days after transduction. Combination of these ten genes produced more ES cell-like colonies than transduction of all 24 genes did. Withdrawal of individual factors from the ten factor pool was performed to further select reprogramming factors [7]. G418-resistant colonies did not form when either Oct4 or Klf4 was removed. Removal of Sox2 resulted in only a few G418-resistant colonies. Removal of c-Myc led to a flatter, non-ES cell-like morphology even though G418-resistant colonies did appear. Removal of the remaining factors did not affect iPS colony numbers significantly. Combination of the four genes produced a number of G418-resistant colonies similar to that observed with the pool of ten genes. Gene expression and epigenetic profiling demonstrated that the iPS cells are similar, although not identical, to ES cells [7]. The iPS clones are pluripotent, having the ability to differentiate into cell types of all three primary germ layers, ectoderm, mesoderm, and endoderm [7]. This study indicated that Oct4, Klf4, Sox2, and c-Myc play important roles in generation of iPS cells.

Starting from a small library of 24 genes that are known to be critical for pluripotency, Takahashi and Yamanaka identified four genes, Oct4, Sox2, Klf4, and cMyc, that can reprogram somatic fibroblasts into ES cell-like pluripotent cells [7]. However, the iPS cells induced in this study shared many but not all features of ES cells. One possible reason for the incomplete reprogramming is that the selection for reprogramming was performed from the locus of Fbx15, a gene that is expressed in ES cells but is not required for pluripotency.

Three subsequent studies led by Yamanaka, Jaenisch, and Hochedlinger individually investigated whether iPS cells could be better reprogrammed by selecting from a locus known to be essential for pluripotency [8-10]. Activation of the endogenous Oct4 or Nanog, genes that are essential for ES cell self-renewal and pluripotency, was used as a more stringent selection strategy for the isolation of reprogrammed cells [8-10]. Introduction of the four reprogramming factors into reporter lines that have selectable markers under the control of Nanog or Oct4 allowed selection of iPS cells that are more similar to ES cells in both epigenetic profiles and developmental potentials [8-10]. With this approach, the chromatin configuration of somatic cells is re-set in the iPS cells to one that is characteristic of ES cells. In addition to forming teratomas that could differentiate into cell types representing all three germ layers, these cells efficiently generated high-contribution chimeras and some of the chimeras allowed germline transmission [8-10]. Furthermore, the selected iPS cells could be injected into tetraploid blastocysts and make embryos that are composed of only the injected cells [9], which represents the most rigorous test for developmental potential.

Recently, research groups led by Yamanaka, Thomson, Daley, and Plath transferred the seminal work on somatic cell reprogramming from the mouse to human [11-14,26]. By overexpressing the same four transcription factors that were used in mouse (Oct4, Sox2, Klf4, and c-Myc) or using a different combination (Oct4, Sox2, Nanog, and Lin28), each group has successfully induced human somatic fibroblasts into human ES cell-like pluripotent stem cells [11-13,26]. Human ES cells are different from mouse ES cells in many ways [27]. However, recent studies showed that the same four transcription factors (Oct4, Sox2, Klf4, and c-Myc) induced pluripotent cells in both human and mouse [9-11,13,26,28-30], suggesting that the transcriptional network that is essential for pluripotency is common in human and mouse.

In the study by Daley's group, Oct4, Sox2, Klf4 and c-Myc were introduced into human embryonic fibroblasts. iPS cells with ES cell-like morphology were identified with a reprogramming efficiency of about 0.1% [13]. Oct4 and Sox2 were shown to be essential for reprogramming, while Myc and Klf4 enhanced the efficiency of iPS colony formation [13]. Furthermore, supplement of the catalytic subunit of human telomerase, hTERT, and SV40 large T antigen to the four reprogramming factors increased the efficiency of reprogramming from human postnatal fibroblasts [13].

Direct reprogramming of somatic cells provides great opportunity to create patient-specific pluripotent stem cells. However, including c-Myc, a known oncogene, in the reprogramming cocktail is worrisome. Indeed, many iPS cell-derived mice developed tumors due to reactivation of the c-Myc retrovirus [8]. The study led by Thomson showed that, four factors with a different combination, Oct4, Sox2, Nanog, and Lin28, are able to reprogram human somatic cells to pluripotent stem cells without c-Myc [12]. These human iPS cells have normal karyotypes, exhibit telomerase activity, express cell surface markers and genes that are characteristic of human ES cells, and maintain developmental potential to differentiate into cell types of all three germ layers. This approach also allowed reprogramming of both fetal and postnatal fibroblasts [12], similar to the Yamanaka approach [11].

More recently, studies led by Yamanaka and Jaenisch also demonstrated independently that generation of iPS cells can be achieved without c-Myc [31,32]. Using three factors, Oct4, Sox2, and Klf4, both groups were able to reprogram fibroblasts into pluripotent stem cells, although reprogramming is delayed with lower efficiency [31,32]. These studies suggest that one of the functions of c-Myc in reprogramming is to enhance proliferation, thus allowing accelerated reprogramming with higher efficiency. The generation of iPS cells without c-Myc oncogene represents a big progress toward safer iPS cell production.

Reprogramming is a gradual process with events occurring in a sequential order [29,33]. Expression of the four transduced reprogramming factors, Oct4, Sox2, c-Myc, and Klf4, is required for at least 8 days, up to 12 to 16 days, before cells enter a self-sustaining pluripotent state by activating endogenous pluripotency factors [29,33]. Endogenous pluripotency markers are expressed sequentially during reprogramming. Expression of endogenous Oct4 and Nanog genes, two essential pluripotency regulators, only occurs late in the process [29,33].

In addition to fibroblasts, iPS cells can also be generated from lineage-committed or terminally differentiated cells now. The four transcription factors, Oct4, Sox2, c-Myc, and Klf4, allowed generation of iPS cells from mouse primary hepatocytes and gastric epithelial cells [28]. Omission of Myc from the cocktail only decreased the reprogramming efficiency 20 to 40%. Moreover, generation of these iPS cells is independent of retroviral integration sites, suggesting that viral integration is dispensable for reprogramming [28]. The same four factor cocktail also allowed reprogramming of immature B lymphocytes. However, reprogramming mature B cells required additional mechanisms, either ectopic expression of the myeloid transcription factor CCAAT/enhancer-binding protein α (C/EBP α) or knockdown of the B cell transcription factor

Pax5 [30]. These studies provide proof-of-principle for direct reprogramming of terminally differentiated adult cells to pluripotency.

Conclusions

Expression of four transcription factors proved to be a robust method to induce reprogramming of somatic cells to a pluripotent state. These studies have opened a new avenue to generate patient- and disease-specific pluripotent stem cells. Human iPS cells will be useful for understanding disease mechanisms, for drug screening and toxicology, and for regenerative medicine in the future. Indeed, the therapeutic potential of iPS cells has been demonstrated in mouse models of sickle cell anemia and Parkinson's disease as proof-of-principle [34,35]. However, the use of retrovirus transduction to introduce reprogramming factors and the use of oncogenes represent serious barriers to the eventual use of iPS cells for therapeutic application in human. While recent reports of reprogramming without c-Myc represent significant progress toward reducing the tumorigenic potential of iPS cells, finding new ways to introduce reprogramming factors to avoid the use of retroviral vectors remains a challenge. Using transient gene expression vectors for gene delivery, introducing reprogramming factors by protein transduction, and ultimately, activating endogenous pluripotency regulators by small molecules, will lead to the generation of genetically unmodified iPS cells for clinical use. Meanwhile, much work is needed to understand the molecular pathways of reprogramming in order to achieve reprogramming without gene transfer. Further challenges include improving the efficiency of the reprogramming process and developing robust differentiation protocols for human iPS cells in order to apply iPS cells for regenerative medicine. In addition to technical challenges, ethical concerns of reproductive cloning also apply to iPS cells. With rapid progress in iPS cell generation, any somatic cells have the potential to be used to clone an individual. Therefore, proper regulation regarding the generation and usage of human iPS cells will be employed to avoid misuses of this technology. Despite the challenges, direct reprogramming of somatic cells clearly open a new era for stem cell biology. It promises to provide new tools for drug discovery and new hope for stem cell therapies

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Abbreviations

ES	embryonic stem cells
iPS	induced pluripotent stem cells

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Table 1

Progress in iPS cell generation

Reprogramming factors, selection markers, the species and type of somatic cells used for reprogramming are listed. The phenotype of reprogramming and references of the studies are also included.

Reprogramming Factors used	Selection Markers	Species	Somatic Cells Used	Phenotype of Reprogramming	References
Oct4, Sox2, c-Myc, Klf4	Fbx15- β geo	Mouse	Embryonic fibroblasts or adult tail-tip fibroblasts	Incomplete reprogramming	Takahashi and Yamanaka, 2006 [7]
Oct4, Sox2, c-Myc, Klf4	Nanog-GFP-IRES-puro ^R	Mouse	Embryonic fibroblasts	Similar to ES cells, chimeras were obtained with germ line transmission	Okita et al 2007 [8]
Oct4, Sox2, c-Myc, Klf4	Oct4-neo ^R or Nanog-neo ^R	Mouse	Embryonic fibroblasts or adult tail-tip fibroblasts	Similar to ES cells, chimeras were formed with germ line transmission	Wernig et al 2007 [9]
Oct4, Sox2, c-Myc, Klf4	Nanog-GFP-IRES-puro ^R	Mouse	Embryonic fibroblasts or adult tail-tip fibroblasts	Similar to ES cells, can form viable chimeras with germ line transmission	Maherali et al 2007 [10]
Oct4, Sox2, c-Myc, Klf4	Morphology	Human	Adult dermal fibroblast, adult fibroblast-like synoviocytes, neonate fibroblasts	Similar to human ES cells	Takahashi et al 2007 [11]
Oct4, Sox2, Nanog, Lin28	Oct4-neo ^R Morphology	Human	Fetal fibroblasts, newborn foreskin fibroblasts	Similar to human ES cells	Yu et al 2007 [12]
Oct4, Sox2, c-Myc, Klf4	Oct4-neo ^R Morphology	Human	Fetal fibroblasts, fetal lung fibroblasts, fetal skin fibroblasts	Similar to human ES cells	Park et al 2007 [13]
Oct4, Sox2, c-Myc, Klf4, hTERT, SV40 large T	Morphology	Human	Neonatal foreskin fibroblasts, adult mesenchymal stem cells, adult dermal fibroblasts	Similar to human ES cells	Park et al 2007 [13]
Oct4, Sox2, Klf4, c-Myc	Morphology	Human	Human neonatal foreskin fibroblasts	Similar to human ES cells	Lowry et al 2008 [26]
Oct4, Sox2, Klf4	Nanog-GFP-IRES-puro ^R , Fbx15- β geo, Morphology	Mouse & human	Mouse embryonic fibroblasts and adult tail-tip fibroblasts, human dermal fibroblasts	More specific reprogramming with lower efficiency	Nakagawa et al 2008 [31]

Reprogramming Factors used	Selection Markers	Species	Somatic Cells Used	Phenotype of Reprogramming	References
Oct4, Sox2, Klf4	Oct4-neo ^R or Nanog-neo ^R	Mouse	Mouse embryonic fibroblasts	Delayed reprogramming with lower efficiency	Wernig et al 2008 [32]
Oct4, Sox2, c-Myc, Klf4	Fbx15-βgeo	Mouse	Primary hepatocytes and gastric epithelial cells	Similar to ES cells. Chimeras were obtained with germ line transmission	Aoi et al 2008 [28]
Oct4, Sox2, c-Myc, Klf4	Oct4-GFP, Nanog-GFP	Mouse	Mouse embryonic fibroblasts	iPS cells were generated gradually with events occurring in a sequential order	Brambrink et al 2008 [29]
Oct4, Sox2, c-Myc, Klf4	Oct4-GFP, Sox2-GFP	Mouse	Mouse embryonic fibroblasts or newborn tail-tip fibroblasts	Pluripotency markers are expressed sequentially during reprogramming	Stadtfield et al 2008 [33]
Oct4, Sox2, c-Myc, Klf4	Nanog-GFP	Mouse	Nonterminally differentiated B lymphocytes	Similar to ES cells. Adult chimeras with germline transmission.	Hanna et al 2008 [30]
Oct4, Sox2, c-Myc, Klf4, C/EBPα, or KD of Pax5	Nanog-GFP	Mouse	Mature B cells	Similar to ES cells. Adult chimeras with germline transmission.	Hanna et al 2008 [30]