

Induced Pluripotent Stem Cells, New Tools for Drug Discovery and New Hope for Stem Cell Therapies

Yanhong Shi*

Division of Neurosciences, and the Center for Gene Expression and Drug Discovery, Beckman Research Institute of City of Hope, 1500 E. Duarte Rd., Duarte, CA, 91010, USA

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 have 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 have revealed a remarkable degree of similarity between these two pluripotent stem cell types. This mini-review summarizes these ground-breaking studies. These advances in reprogramming will enable the creation of patient-specific stem cell lines to study various disease mechanisms. The cellular models created will provide valuable tools for drug discovery. Furthermore, this reprogramming system provides great potential to design customized patient-specific stem cell therapies with economic 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 currently the main source for producing human pluripotent stem cells that are genetically unmodified. 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 for embryos or eggs will solve the technical and ethical problems.

With studies led by Yamanaka, Jaenisch, Hochedlinger, Thomson, and Daley, an important revolution in stem cell research has been 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 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, are involved 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. 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 the generation of iPS cells.

Gene expression and epigenetic profiling demonstrated that the iPS cells are similar, but not identical, to ES cells [7]. 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 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. 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]. This is a test that represents the most rigorous standard for developmental potential.

*Address correspondence to this author at the Division of Neurosciences, and the Center for Gene Expression and Drug Discovery, Beckman Research Institute of City of Hope, 1500 E. Duarte Rd., Duarte, CA, 91010, USA; E-mail: yshi@coh.org

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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 2007 [10]
Oct4, Sox2, c-Myc, Klf4	Morphology	Human	Adult dermal fibroblast, adult fibroblast-like synoviocytes, neonate fibroblasts	Similar to human ES cells	Takahashi <i>et al.</i> 2007 [11]
Oct4, Sox2, Nanog, Lin28	Oct4-neo ^R Morphology	Human	Fetal fibroblasts, newborn foreskin fibroblasts	Similar to human ES cells	Yu <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 2007 [13]
Oct4, Sox2, Klf4, c-Myc	Morphology	Human	Human neonatal foreskin fibroblasts	Similar to human ES cells	Lowry <i>et al.</i> 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 <i>et al.</i> 2008 [31]
Oct4, Sox2, Klf4	Oct4-neo ^R or Nanog-neo ^R	Mouse	Mouse embryonic fibroblasts	Delayed reprogramming with lower efficiency	Wernig <i>et al.</i> 2008 [32]
Oct4, Sox2, c-Myc, Klf4	Fbx15- β geo	Mouse	Primary hepatocytes and gastric epithelial cells	Similar to ES cells. Chimeras were obtained with germ line transmission	Aoi <i>et al.</i> 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 <i>et al.</i> 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	Stadtfeld <i>et al.</i> 2008 [33]
Oct4, Sox2, c-Myc, Klf4	Nanog-GFP	Mouse	Nonterminally differentiated B lymphocytes	Similar to ES cells. Adult chimeras with germline transmission.	Hanna <i>et al.</i> 2008 [30]
Oct4, Sox2, c-Myc, Klf4, C/EBP α , or KD of Pax5	Nanog-GFP	Mouse	Mature B cells	Similar to ES cells. Adult chimeras with germline transmission	Hanna <i>et al.</i> 2008 [30]

Recently, research groups led by Yamanaka, Thomson, Daley, and Plath transferred the seminal work on somatic cell reprogramming from mouse to human [11-14, 26]. By overexpressing the same four transcription factors that were used in mice (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, supplementing the four reprogramming factors with the catalytic subunit of human telomerase, hTERT, and SV40 large T antigen, 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, in-

cluding 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 a different combination of four factors, Oct4, Sox2, Nanog, and Lin28, is 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 demonstrated independently that generation of iPS cells could 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 step towards 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, and 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 now be generated from lineage-committed or terminally differentiated cells. 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.

CONCLUSION

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 applications 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 while avoiding 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 pluripo-

tency 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, regulation of the generation and usage of human iPS cells must be addressed. Despite the challenges, direct reprogramming of somatic cells clearly opens a new era for stem cell biology. It promises to provide new tools for drug discovery and new hope for stem cell therapies.

ACKNOWLEDGMENTS

We thank Dr Chunnian Zhao for critical reading of the manuscript, Jill Brantley and Jennifer Finnie for editing and proof-reading of the manuscript. Y.S. is a Kimmel Scholar. This work was supported by Whitehall Foundation, Margret E. Early Medical Trust, James S. McDonnell Foundation, and NIH NINDS.

ABBREVIATIONS

ES = Embryonic stem cells
iPS = Induced pluripotent stem cells

REFERENCES

- Thomson, J. A.; Itskovitz-Eldor, J.; Shapiro, S. S.; Waknitz, M. A.; Swiergiel, J. J.; Marshall, V. S.; Jones, J. M. Embryonic stem cell lines derived from human blastocysts. *Science* **1998**, *282*, 1145-1147.
- Wakayama, T.; Tabar, V.; Rodriguez, I.; Perry, A. C.; Studer, L.; Mombaerts, P. Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer. *Science* **2001**, *292*, 740-743.
- Eggle, D.; Rosains, J.; Birkhoff, G.; Eggan, K. Developmental reprogramming after chromosome transfer into mitotic mouse zygotes. *Nature* **2007**, *447*, 679-685.
- Tada, M.; Takahama, Y.; Abe, K.; Nakatsuji, N.; Tada, T. Nuclear reprogramming of somatic cells by *in vitro* hybridization with ES cells. *Curr. Biol.* **2001**, *11*, 1553-1558.
- Cowan, C. A.; Atienza, J.; Melton, D. A.; Eggan, K. Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science* **2005**, *309*, 1369-1373.
- Jaenisch, R. Human cloning - the science and ethics of nuclear transplantation. *N. Engl. J. Med.* **2004**, *351*, 2787-2791.
- Takahashi, K.; Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **2006**, *126*, 663-676.
- Okita, K.; Ichisaka, T.; Yamanaka, S. Generation of germline-competent induced pluripotent stem cells. *Nature* **2007**, *448*, 313-317.
- Wernig, M.; Meissner, A.; Foreman, R.; Brambrink, T.; Ku, M.; Hochedlinger, K.; Bernstein, B. E.; Jaenisch, R. *In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* **2007**, *448*, 318-324.
- Maherali, N.; Sridharan, R.; Xie, W.; Utikal, J.; Eminli, S.; Arnold, K.; Stadtfeld, M.; Yachechko, R.; Tchieu, J.; Jaenisch, R.; Plath, K.; Hochedlinger, K. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell* **2007**, *1*, 55-70.
- Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K.; Yamanaka, S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **2007**, *131*, 861-872.
- Yu, J.; Vodyanik, M. A.; Smuga-Otto, K.; Antosiewicz-Bourget, J.; Frane, J. L.; Tian, S.; Nie, J.; Jonsdottir, G. A.; Ruotti, V.; Stewart, R.; Slukvin, I.; Thomson, J. A. Induced pluripotent stem cell lines derived from human somatic cells. *Science* **2007**, *318*, 1917-1920.
- Park, I. H.; Zhao, R.; West, J. A.; Yabuuchi, A.; Huo, H.; Ince, T. A.; Lerou, P. H.; Lensch, M. W.; Daley, G. Q. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* **2008**, *451*, 141-146.
- Zaehres, H.; Scholer, H. R. Induction of pluripotency: from mouse to human. *Cell* **2007**, *131*, 834-835.
- Nichols, J.; Zevnik, B.; Anastassiadis, K.; Niwa, H.; Klewe-Nebenius, D.; Chambers, I.; Scholer, H.; Smith, A. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell* **1998**, *95*, 379-391.

- [16] Niwa, H.; Miyazaki, J.; Smith, A. G. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nat. Genet.* **2000**, *24*, 372-376.
- [17] Avilion, A. A.; Nicolis, S. K.; Pevny, L. H.; Perez, L.; Vivian, N.; Lovell-Badge, R. Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev.* **2003**, *17*, 126-140.
- [18] Chambers, I.; Colby, D.; Robertson, M.; Nichols, J.; Lee, S.; Tweedie, S.; Smith, A. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell* **2003**, *113*, 643-655.
- [19] Mitsui, K.; Tokuzawa, Y.; Itoh, H.; Segawa, K.; Murakami, M.; Takahashi, K.; Maruyama, M.; Maeda, M.; Yamanaka, S. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell* **2003**, *113*, 631-642.
- [20] Matsuda, T.; Nakamura, T.; Nakao, K.; Arai, T.; Katsuki, M.; Heike, T.; Yokota, T. STAT3 activation is sufficient to maintain an undifferentiated state of mouse embryonic stem cells. *EMBO J.* **1999**, *18*, 4261-4269.
- [21] Niwa, H.; Burdon, T.; Chambers, I.; Smith, A. Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3. *Genes Dev.* **1998**, *12*, 2048-2060.
- [22] Cartwright, P.; McLean, C.; Sheppard, A.; Rivett, D.; Jones, K.; Dalton, S. LIF/STAT3 controls ES cell self-renewal and pluripotency by a Myc-dependent mechanism. *Development* **2005**, *132*, 885-896.
- [23] Li, Y.; McClintick, J.; Zhong, L.; Edenberg, H. J.; Yoder, M. C.; Chan, R. J. Murine embryonic stem cell differentiation is promoted by SOCS-3 and inhibited by the zinc finger transcription factor Klf4. *Blood* **2005**, *105*, 635-637.
- [24] Kielman, M. F.; Rindapaa, M.; Gaspar, C.; van Poppel, N.; Breukel, C.; van Leeuwen, S.; Taketo, M. M.; Roberts, S.; Smits, R.; Fodde, R. Apc modulates embryonic stem-cell differentiation by controlling the dosage of beta-catenin signaling. *Nat. Genet.* **2002**, *32*, 594-605.
- [25] Sato, N.; Meijer, L.; Skaltsounis, L.; Greengard, P.; Brivanlou, A. H. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. *Nat. Med.* **2004**, *10*, 55-63.
- [26] Lowry, W. E.; Richter, L.; Yachechko, R.; Pyle, A. D.; Tchieu, J.; Sridharan, R.; Clark, A. T.; Plath, K. Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 2883-2888.
- [27] Rao, M. Conserved and divergent paths that regulate self-renewal in mouse and human embryonic stem cells. *Dev. Biol.* **2004**, *275*, 269-286.
- [28] Aoi, T.; Yae, K.; Nakagawa, M.; Ichisaka, T.; Okita, K.; Takahashi, K.; Chiba, T.; Yamanaka, S. Generation of Pluripotent Stem Cells from Adult Mouse Liver and Stomach Cells. *Science* **2008**, *321*, 699-702.
- [29] Brambrink, T.; Foreman, R.; Welstead, G. G.; Lengner, C. J.; Wernig, M.; Suh, H.; Jaenisch, R. Sequential expression of pluripotency markers during direct reprogramming of mouse somatic cells. *Cell Stem Cell* **2008**, *2*, 151-159.
- [30] Hanna, J.; Markoulaki, S.; Schorderet, P.; Carey, B. W.; Beard, C.; Wernig, M.; Creighton, M. P.; Steine, E. J.; Cassady, J. P.; Foreman, R.; Lengner, C. J.; Dausman, J. A.; Jaenisch, R. Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. *Cell* **2008**, *133*, 250-264.
- [31] Nakagawa, M.; Koyanagi, M.; Tanabe, K.; Takahashi, K.; Ichisaka, T.; Aoi, T.; Okita, K.; Mochizuki, Y.; Takizawa, N.; Yamanaka, S. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat. Biotechnol.* **2008**, *26*, 101-106.
- [32] Wernig, M.; Meissner, A.; Cassady, J. P.; Jaenisch, R. c-Myc is dispensable for direct reprogramming of mouse fibroblasts. *Cell Stem Cell* **2008**, *2*, 10-12.
- [33] Stadtfeld, M.; Maherali, N.; Breault, D. T.; Hochedinger, K. Defining molecular cornerstones during fibroblast to iPS cell reprogramming in mouse. *Cell Stem Cell* **2008**, *2*, 230-240.
- [34] Wernig, M.; Zhao, J. P.; Pruszak, J.; Hedlund, E.; Fu, D.; Soldner, F.; Broccoli, V.; Constantine-Paton, M.; Isacson, O.; Jaenisch, R. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 5856-5861.
- [35] Hanna, J.; Wernig, M.; Markoulaki, S.; Sun, C. W.; Meissner, A.; Cassady, J. P.; Beard, C.; Brambrink, T.; Wu, L. C.; Townes, T. M.; Jaenisch, R. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science* **2007**, *318*, 1920-1923.