Cell Type-Specific Expression Profile and Signaling Requirements in Early Hematopoietic Reprogramming

Lan Kang,^{1,3,*} Jun Wu,^{2,*} Yu Tao,^{3,4,*} Haolan Li,¹ Xiaolei Liu,³ Shaorong Gao,^{3,5} and Qian-Fei Wang²

Hematopoietic cells represent an attractive starting cell type for induced pluripotent stem (iPS) cells induction, yet the molecular mechanisms in hematopoietic reprogramming are poorly defined. In this study, we showed that long-term hematopoietic stem cells are more amenable for iPS cells induction among several hematopoietic stem and progenitor cell (HSPC) populations, and that this is accompanied by an earlier induction of the transcriptional program that is involved in the promotion of macromolecule metabolism and cell proliferation. Notably, we identified multiple signaling pathways that exhibited distinct expression patterns in HSPCs compared to that of fibroblasts, which is the most commonly used model for probing the somatic reprogramming process. We further experimentally confirmed the differential requirements of the Wnt/ β -catenin and transforming growth factor-beta (TGF- β) signaling pathways in these two cell types. These data demonstrate that hematopoietic cells have a cell-type specific transcriptional program and possess unique signaling requirements in the early phase of reprogramming.

Introduction

BY ECTOPICALLY EXPRESSING *Oct4*, *Sox2*, *Klf4*, and *c-Myc*, somatic cells can be reprogrammed into induced pluripotent stem (iPS) cells [1]. The successful generation of human and patient-specific iPS cells has raised the possibility of utilizing iPS cells in regenerative medicine, pathological studies, and drug screening for diseased individuals [2].

Although mouse embryonic fibroblasts (MEFs) are the most commonly used model system for investigating the molecular mechanism involved in iPS cell induction, multiple somatic cells with distinct tissue identity, including hematopoietic, neural, liver, and stomach cells, have been successfully reprogrammed to pluripotency [3–5]. Specifically, cells with hematopoietic origin are regarded as an excellent alternative in the generation of iPS cells. While human skin fibroblasts are obtained via an invasive and nonsterile procedure and require 2–3 weeks of expansion before experimentation, hematopoietic cells, especially peripheral blood mononuclear cells, are easily accessible [6]. In addition, skin cells carry more somatic genomic variations due to environmental aggressions, such as ultraviolet irradiation, than cells from inside the body [7].

Understanding the molecular mechanisms involved in reprogramming will address fundamental questions such as cell identity and cell fate decision and improve reprogramming efficiency and quality. Recent studies suggest that reprogram-

ming is a multiphase process [8,9]. The early phase involves inhibition of the original cell identity accompanied by events associated with proliferation, metabolism changes, and cytoskeleton organization. Subsequently, the cells will go through a process of stochastic activation of pluripotency markers and glycolysis (intermediate phase) and activation of the core pluripotency circuitry and silencing of transgenes (late phase) before they reach iPS cell maturation. Despite these insightful findings in MEF reprogramming, much less is known regarding the underlying mechanisms that occur when other cells are employed.

Considering the drastic differences in reprogramming efficiency among various cell types and cells at different differentiation stages [10,11], cell-specific molecular mechanisms may be in operation. In this study, we explored the early events in the reprogramming of hematopoietic stem and progenitor cells (HSPCs). Our results suggested commonalities between fibroblasts and hematopoietic cells and revealed distinct requirements of the Wnt/ β -catenin and transforming growth factor-beta (TGF- β) signaling pathways in these two cell types.

Materials and Methods

Animals

Specific pathogen-free mice were housed in the animal facility of the National Institute of Biological Sciences. All

¹Institute of Cancer Stem Cell (ICSC), Dalian Medical University, Dalian, People's Republic of China.

²Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, People's Republic of China.

³National Institute of Biological Sciences, Beijing, People's Republic of China.

⁴College of Life Sciences, Beijing Normal University, Beijing, People's Republic of China.

⁵School of Life Sciences and Technology, Tongji University, Shanghai, People's Republic of China.

*These authors contributed equally to this work.

studies adhered to procedures that were consistent with the National Institute of Biological Sciences Guide for the care and use of laboratory animals.

Induced pluripotent stem cell induction and all-iPS mice generation

Tet-on-inducible lentiviruses carrying cDNAs of the reprogramming factors were used for iPS induction. All-iPS mice were obtained through tetraploid embryo complementation as previously described [12].

Hematopoietic cell reprogramming

Long-term hematopoietic stem cells (LT-HSCs), short-term hematopoietic stem cells (ST-HSCs), myeloid progenitors (MPs), and lineage negative (Lin⁻) hematopoietic cells were cultured in iPS cell culture medium supplemented with 50 ng/mL stem cell factor (SCF), 20 ng/mL human thrombopoietin (TPO), 10 ng/mL mouse interleukin (IL)-3, 10 ng/mL mouse IL-6, 10 ng/mL mouse Flt-3 ligand, and penicillin/streptomycin (100×; Merk Millipore) in the presence of feeder cells. All cytokines were purchased from Peprotech, if not mentioned specifically. The Gsk-3β inhibitor (CHIR-99021; Selleck) and Tgf-β R1 kinase inhibitor A-83-01 (616454; Merck) were added to the culture medium the same time as iPS induction. A Leukocyte Alkaline Phosphatase Kit (Sigma) was employed for alkaline phosphatase (AP) staining according to the manufacturer's instructions.

RNA sequencing and expression analysis

The single-cell RNA-seq strategy [13] was employed to capture the transcriptomes of 25 hematopoietic cells. An RNA-seq library was prepared using the protocol of NEBNext Singleplex (#E7350) oligos from Illumina. All reads were aligned to the mouse reference genome mm9 from UCSC using TopHat [14]. The rates of unique maps ranged from 43.20% to 54.28% for 18 samples (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/scd), and only unique mapped reads were used for further analyses. Examination of the read distribution showed that most of the reads mapped to exon-CDS (average 47.3%) and exon-UTR3 (30.3%) regions (Supplementary Fig. S1). Cufflinks [15] was used to measure the relative abundance of each transcript using the Ensemble Transcript ID. Differentially expressed genes were identified using DEGseq [16], with $P < 0.001$ and > 3 -fold change as cutoff.

Statistical analysis

Gene functional enrichment analysis was performed using DAVID (<http://david.abcc.ncifcrf.gov/>, GO-BP terms, FDR < 0.05). Canonical pathways, which were collected from the Ingenuity Knowledgebase of Ingenuity Pathway Analysis (IPA, www.ingenuity.com/products/ipa), were used to identify significant signaling pathways. All other statistical analyses were performed using the corresponding R statistical packages.

Data access

The deep sequencing RNA data from this study have been deposited in the NCBI database and are accessible through

GEO series accession number GSE54462 (www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54462).

Detailed description could be found in Supplementary Experimental Procedures section.

Results

Reprogramming of HSPCs

To explore the early molecular events in the reprogramming of HSPCs, we employed a secondary reprogramming system in which reprogramming factors can be induced by doxycycline (Dox) in a genetically homogenous cell population [12,17]. Specifically, we obtained primary iPS cell lines by infecting mouse fibroblasts with Dox-controlled, Tet-on-inducible lentiviruses expressing *Oct4*, *Sox2*, *Klf4*, and *c-Myc*. All-iPS mice were generated by tetraploid embryo complementation using one of these cell lines, LiPS-37 [18]. Therefore, all tissues/cells of these mice carry the expression vector at identical genomic positions and can be induced to express the reprogramming factors by the addition of Dox (Fig. 1A).

To determine whether secondary iPS cells can be obtained from HSPCs, we isolated several hematopoietic stem cell and progenitor populations based on the surface markers LSK, CD34 [19,20], and assisted by CD150 [21] (Fig. 1B). While LT-HSCs (Lin⁻Sca-1⁺c-Kit⁺CD34⁻CD150⁺) reside at the top of the hematopoietic hierarchy and have long-term populating capability, ST-HSCs (Lin⁻Sca-1⁺c-Kit⁺CD34⁺CD150⁻) and MPs (Lin⁻Sca-1⁻c-Kit⁺) are more limited in their potential to sustain hematopoiesis.

All three populations were cultured in iPS cell culture medium supplemented with Dox and the same cytokine cocktail consisting of SCF, TPO, IL-3, IL-6, and Flt-3 ligand for 2 weeks. After picking and expanding single colonies, all three HSPC populations gave rise to iPS colonies and stable iPS lines (Fig. 1C), and immunofluorescence staining showed the Nanog expression in these iPS cells (Fig. 1D).

Overall, more than 100 AP⁺ colonies could be derived from 2,000 starting cells (Fig. 1E). Interestingly, the iPS colonies that were derived from LT-HSCs were obviously larger than those from ST-HSCs and MPs (Fig. 1E), suggesting that these colonies might have formed at an earlier time. In fact, LT-HSCs became adherent to the plate, congregated the earliest, and gave rise to compact colonies as early as day 4 after Dox addition, whereas colony formation of ST-HSCs and MPs required an additional 1–2 days to take place (Fig. 1F and Supplementary Fig. S2). These results suggest that LT-HSCs reprogram faster than ST-HSCs and MPs.

Biological processes involved in early reprogramming of different hematopoietic cells

Based on the observed faster reprogramming in LT-HSCs, we hypothesized that LT-HSCs may have an earlier induction of favorable molecular events that lead to pluripotency. Therefore, we performed single cell RNA-seq [13] to capture the transcriptome from small number of hematopoietic cells.

We identified the transcriptional changes 2 and 4 days after Dox induction on each of the HSPC populations (Supplementary Fig. S3 and Supplementary Experimental Procedures section). Only about 11% and 11.5% of differentially

FIG. 1. Reprogramming of hematopoietic stem and progenitor cells (HSPCs). **(A)** Schematic diagram of secondary reprogramming system based on all-induced pluripotent stem (iPS) mice. **(B)** FACS strategy for long-term hematopoietic stem cells (LT-HSCs; Lin⁻Sca-1⁺c-Kit⁺CD34⁻CD150⁺), short-term hematopoietic stem cells (ST-HSCs; Lin⁻Sca-1⁺c-Kit⁺CD34⁺CD150⁻), and myeloid progenitors (MPs; Lin⁻Sca-1⁻c-Kit⁺). Lin: Gr-1, Ter-119, CD11b, B220, CD3e, CD4, and CD8. **(C)** Morphology of the iPS colonies (upper) and cell lines after propagation (lower) derived from LT-HSCs, ST-HSCs, and MPs, by 2 weeks induction of doxycycline (Dox) controlled O/S/K/M expression. Scale bars represent 100 μ m. **(D)** Immunofluorescence staining showing the protein level of Nanog in the iPS cell lines derived from hematopoietic cells. Scale bars represent 25 μ m. **(E)** Alkaline phosphatase (AP) staining of iPS colonies derived from LT-HSCs, ST-HSCs, and MPs. **(F)** Cell morphology at indicated time point during Dox-induced reprogramming showing the different pace beginning with LT-HSCs, ST-HSCs, and MPs. Scale bars represent 100 μ m. Color images available online at www.liebertpub.com/scd

expressed genes were common in three hematopoietic cells after 2 and 4 days induction respectively, while about 60% of the differentially expressed genes had cell specificity (Fig. 2A).

Differentially upregulated genes in Dox-induced LT-HSCs at days 2 and 4 were enriched for cell cycle, RNA/DNA processing, mitochondria, metabolism and biosynthetic processes, which are known molecular pathways that facilitate reprogramming (Fig. 2B and Supplementary Fig. S4 and Supplementary Experimental Procedures section). These results corroborate with earlier findings that molecular events related to proliferation are implemented at the earliest stages of reprogramming [9]. In contrast, these proliferation- and metabolism-related biological processes were more limited in Dox-treated ST-HSCs and MPs (Fig. 2B and Supplementary Fig. S4). ST-HSCs exhibited increased expression of genes related to filament depolymerization and protein complex assembly, while MPs exhibited significant induction of gene expression in organelle organization, cellular development, cell death, and apoptosis (Fig. 2B and Supplementary Fig. S5), which is a reported barrier of reprogramming [22].

Together, these data suggest that though LT-HSCs were the most quiescent cells among these cell types but responded quickly to the induction in early stage, and all three HSPC populations exhibited various degrees of metabolic reorganization in setting the stage for reprogramming.

Silencing of hematopoietic tissue-specific genes, but not the mesenchymal-to-epithelial transition, was an early feature of HSPC reprogramming

The mesenchymal-to-epithelial transition (MET) was identified as a hallmark in the initiation phase of MEF re-

programming and is characterized by downregulation of mesenchymal genes and induction of epithelial-associated genes [23,24]. To explore whether MET also occur as early event in HSPC reprogramming, we first examined the levels of expression of the key mesenchymal regulators and fibroblast markers *Snail*, *Slug*, *Zeb1*, *Zeb2*, *Cdh2*, and *Thy1*. While high-level expression of all six genes were significantly downregulated as early as day 2 during fibroblast reprogramming, we could barely detect expression of the same set of genes in uninduced, as well as 2- and 4-day Dox-treated HSPCs (Fig. 3A). Considering the mesenchymal property of fibroblasts, we reasoned that this MET phenomenon emphasizes the suppression of the expression of genes specific to the starting cell type of reprogramming.

Therefore, we tested whether the expression of hematopoietic-specific genes were specifically downregulated in the process of HSPC reprogramming. To define hematopoietic/HSPC-specific genes, we compared the expression profile of each of the HSPC populations with that of fibroblast cells obtained from the skin of all-iPS mice in the absence of Dox treatment (day 0) (Supplementary Fig. S3 and Supplementary Experimental Procedures section).

Compared to fibroblasts, 718 highly expressed genes were shared by the three HSPC cell populations ($P < 0.001$ and fold change (FC) > 3 , Fig. 3B, upper left). Approximately 90% of the differentially expressed, HSPC-specific genes (see Supplementary Experimental Procedures section) were downregulated after Dox treatment (Fig. 3B, lower left and Supplementary Fig. S6). These genes were enriched in the immune response, leukocyte activation, and leukocyte differentiation (Supplementary Table S2). Consistent with the RNA-seq data, the expression of hematopoietic key

FIG. 2. Expression profile analysis of early reprogramming of different HSPCs. **(A)** Venn diagram showing differentially changed genes among LT-HSC, ST-HSC, and MP cells after 2 and 4 days Dox induction. **(B)** Heat map showing results of gene functional annotation analyses using upregulated genes in 2 and 4 days after Dox induction on each of the HSPC populations (LT-HSC, ST-HSC, and MP). Blank indicates that genes were not enriched in this GO-term. Color images available online at www.liebertpub.com/scd

regulators, such as *Gata2*, *PU.1*, *Myb*, and *Lmo2* were markedly decreased after the induction of reprogramming, as detected by real-time PCR (Fig. 3C). Compared to HSPCs, 1,314 genes were highly expressed in fibroblasts (fibroblast-specific genes, Fig. 3B, upper right), and 88% of the differentially expressed, fibroblast-specific genes after Dox-treatment were downregulated and

mostly enriched in cell adhesion, biological adhesion, and extracellular matrix, among others (Fig. 3B, lower right and Supplementary Fig. S6 and Supplementary Table S2). Together, these results demonstrated that downregulation of tissue-specific genes was a common event in the initial phase of reprogramming of fibroblasts and hematopoietic cells.

Differential requirements of Wnt/ β -catenin and TGF- β signaling in reprogramming of HSPCs and fibroblasts

The identification of critical signaling events in reprogramming provides biological insights into this complex process and offers attractive targets when using small molecules to improve iPS induction. To search for molecular pathways that are specifically involved in the early phase of iPS induction of HSPCs, we identified differentially expressed genes after 2 days of Dox treatment in HSPCs and fibroblasts, respectively (Fig. 4A and Supplementary Fig. S3 and Supplementary Experimental Procedures section).

Using signaling pathway enrichment analysis (IPA), we found several pathways that were upregulated in HSPCs but downregulated in fibroblasts (Fig. 4B and Supplementary Table S3). Among these pathways, Wnt/ β -catenin and TGF- β have been previously shown to be important signaling pathways in stem cells and reprogramming. The expression changes during hematopoietic cells reprogramming were confirmed by real-time PCR of key genes, including *Fzd1*, *Lef1*, and *Gsk3b* in the Wnt pathway and *Tgfb2* and *Smad4* in the TGF- β pathway (Fig. 4C).

We further performed functional tests to determine the effects of these two pathways in the reprogramming of HSPCs (Lin⁻ hematopoietic cells) and fibroblasts (see Supplementary Experimental Procedures section). Consistent with previous findings [25,26], the inhibition of TGF- β signaling via A-83-01, a small molecule that specifically inhibits TGF- β type I receptor ALKs (activin receptor-like kinases), remarkably promoted reprogramming in fibroblasts (FC=2.73, $P<0.05$) (Fig. 5A, left). This result is consistent with the notion that TGF- β signaling could induce the mesenchymal phenotype [27], which is a barrier to MEF reprogramming. In sharp contrast, A-83-01 minimally affected iPS induction in HSPCs (FC=0.71, $P<0.05$) (Fig. 5B, right), demonstrating the differential requirements of TGF- β signaling in hematopoietic cells and fibroblasts.

To explore whether the different effects of TGF- β inhibitor on reprogramming are depending on different starting contexts, we investigated the initial expression levels of TGF- β pathway genes in HSPCs and fibroblasts with RNA-seq data. The expression level of TGF- β pathway genes in starting cells of fibroblasts was much higher than that of HSPCs ($P<0.01$, Supplementary Fig. S7). This expression difference tended to be eliminated after reprogramming ($P=0.6$, Supplementary Fig. S7).

FIG. 5. Wnt/ β -catenin and TGF- β signaling profile in HSPCs and fibroblasts reprogramming. (A) Relative AP⁺ colony number (upper) and AP staining (lower) of iPS colonies that were formed from fibroblasts (left) and HSPCs (right) showing the effects of the Gsk3 β and TGF- β inhibitors on reprogramming. AP⁺ colony number of each sample was normalized to the control group. (B) Relative AP⁺ colony number (upper) of iPS colonies that were formed from HSPC. Gsk3 β inhibitor was employed for the period of 1–12 days (1–12), 1–6 days (1–6), and 7–12 days (7–12) during reprogramming. (C) Relative AP⁺ colony number (upper) and AP staining (lower) of iPS colonies that were formed from PBMCs showing the effects of the Gsk3 β and TGF- β inhibitors on reprogramming. AP⁺ colony number of each sample was normalized to the control group. Error bars represent the mean $\pm$ SD. Color images available online at www.liebertpub.com/scd

Furthermore, we found that *Alk4* (*Acvr1b*), one of the targets of A-83-01, showed significantly lower expression in hematopoietic cells than in fibroblasts (Supplementary Fig. S8). Thus, differential expression of *Alk4* may render hematopoietic cells unresponsive to the inhibitor. Interestingly, *c-Myc*, which is a key gene known to significantly impact TGF- β signaling, was expressed at a higher level in hematopoietic cells than in fibroblasts, as shown by our own RNA-seq data (Supplementary Fig. S9) and an independent public dataset (GSE36290) [28]. This result is supportive of earlier findings in which a TGF- β inhibitor much more significantly promoted reprogramming in the absence than in the presence of *c-Myc* [25,26].

Furthermore, the enhancement of the Wnt pathway by inhibition of Gsk3 β (CHIR-99021), a strong negative regulator of the Wnt pathway, significantly increased the efficiency of the AP⁺ iPS colonies generated from HSPCs (FC = 2.04, $P < 0.05$) (Fig. 5A, right). Addition of the Gsk3 β inhibitor in the early (1–6 day) or late (7–12 day) phase of the reprogramming had only moderate impact (Fig. 5B), suggesting that Gsk3 β inhibition is required during the entire course of reprogramming to promote hematopoietic cells reprogramming. Consistent with the finding in HSPCs, enhanced Wnt signaling via inhibition of Gsk3 β significantly facilitated reprogramming of monocytes isolated from peripheral blood, a convenient source for clinical applications of iPS technology (FC = 1.91, $P < 0.05$) (Fig. 5C). However, treatment of fibroblasts with the Gsk3 β inhibitor had a moderate effect on iPS colony formation (FC = 1.53, $P < 0.05$) (Fig. 5A, left). These results identified Wnt as a strong stimulator in the early phase of reprogramming for HSPCs and in mature monocytes.

Discussion

The induction of iPS cells is a low-efficiency, time-consuming process, and its molecular mechanism is not well understood. While progress has been made regarding molecular insights into MEF reprogramming, less is known about the underlying mechanisms when other somatic tissues, including HSPCs, are reprogrammed to pluripotency. Our study is the first to examine the early events in HSPC reprogramming. Similar to the downregulation of mesenchymal-associated genes in fibroblasts, the repression of hematopoiesis-associated genes was featured in hematopoietic reprogramming. Significantly increased gene expression that was associated with cell cycle, RNA processing, mitochondria, and metabolism was observed when HSPCs with different self-renewal potentials were induced to form iPS cells.

Our study also provided evidence that LT-HSCs, when compared with several HSPC populations, were more amenable to reprogramming with higher iPS cell induction velocity, and this was accompanied by an earlier transcriptional induction of metabolic and proliferative genes. This result is consistent with the recent finding that nonstochastic reprogramming was observed in hematopoietic cells [29].

An earlier study also suggested that HSPCs are easier to reprogram than differentiated cells and that hematopoietic progenitor cells showed higher reprogramming efficiency than HSCs [11]. It is worth noting that the iPS induction efficiency is traditionally presented simply as the ratio of the

number of iPS colonies to the number of starting cells. For adherent cells, the progenies of single-starting cells will give rise to a single colony. However, for floating cells, all progenies of single-starting cells have the possibility of giving rise to independent colonies before they become adherent to the plate. Therefore, this counting method may be largely influenced by cell proliferation and the time that is required for the cells to become adherent and iPS cells. This might also explain why hematopoietic progenitor cells, which are more active in proliferation, had a slower reprogramming pace, yet showed higher reprogramming efficiency than HSCs [11].

The Wnt/ β -catenin pathway has been shown to participate in somatic reprogramming. In the absence of *c-Myc* [22] or *Sox2* [30], Wnt signaling can facilitate the generation of iPS cells. However, in the presence of all four factors, Wnt stimulation had modest to no effect on the reprogramming of MEFs to pluripotency [22,31]. These reports are consistent with our finding that showed that the activation of the Wnt pathway by a Gsk3 β inhibitor had only a modest impact on the reprogramming of fibroblasts (Fig. 5A, left). In contrast, inhibition of Gsk3 β significantly promoted the induction of iPS cells from hematopoietic cells (Fig. 5A, right).

Interestingly, a recent study demonstrated a biphasic role of Wnt signaling, in which the key Wnt signaling genes *Tcf3* and *Tcf4* played a different role in the early and late stages of somatic MEF reprogramming [31]. In our gene expression analysis, we also noticed a stronger transcriptional induction of these genes during early HSPC reprogramming compared with that of fibroblasts (data not shown). These findings warrant further investigation on how the cell type-specific expression of key genes influences the distinct requirement of Wnt signaling.

TGF- β signaling can actively maintain the mesenchymal phenotype in fibroblasts and is a well-known inducer of the EMT, the reverse process of MET. Consistently, TGF- β inhibition enhanced reprogramming of MEFs by inducing the MET [24,32]. In support of these earlier findings, our data showed that the TGF- β inhibitor A-83-01 increased the efficiency of iPS colony formation from fibroblasts. However, this inhibitor showed no positive effects in HSPC reprogramming. This difference may result from different starting contexts, as we showed, HSPCs exhibited a completely opposite expression pattern of the key genes that are known to positively affect MEF reprogramming, including low-expressed mesenchymal genes and key TGF- β signaling genes such as *Alk4* and a higher level of expression of *c-Myc*. These data suggest that the cell type-specific context, which is defined by the unique expression pattern of key genes in the starting cells, may have a strong impact on the signaling requirement in the early phase of reprogramming.

Acknowledgments

We thank Professor Rudolf Jaenisch for generously supplying the lentivirus vectors containing the cDNAs of the four transcription factors, Zhenyu Ju and Tao Cheng for help with the hematopoietic cell sorting strategy, and Fuchou Tang for the introduction of single-cell transcriptome analysis. This project was supported by the National

Natural Science Foundation of China (grants 31371512, 81322029, and 81300438) and the “Strategic Priority Research Program” of the Chinese Academy of Sciences XDA01010305.

Author Disclosure Statement

No competing financial interests exist.

References

1. Takahashi K and S Yamanaka. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126:663–676.
2. Park IH, N Arora, H Huo, N Maherali, T Ahfeldt, A Shimamura, MW Lensch, C Cowan, K Hochedlinger and GQ Daley. (2008). Disease-specific induced pluripotent stem cells. *Cell* 134:877–886.
3. Hanna J, M Wernig, S Markoulaki, CW Sun, A Meissner, JP Cassidy, C Beard, T Brambrink, LC Wu, TM Townes and R Jaenisch. (2007). Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science* 318:1920–1923.
4. Kim JB, H Zaehres, G Wu, L Gentile, K Ko, V Sebastiano, MJ Arauzo-Bravo, D Ruau, DW Han, M Zenke and HR Scholer. (2008). Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. *Nature* 454:646–650.
5. Aoi T, K Yae, M Nakagawa, T Ichisaka, K Okita, K Takahashi, T Chiba and S Yamanaka. (2008). Generation of pluripotent stem cells from adult mouse liver and stomach cells. *Science* 321:699–702.
6. Loh YH, S Agarwal, IH Park, A Urbach, H Huo, GC Heffner, K Kim, JD Miller, K Ng and GQ Daley. (2009). Generation of induced pluripotent stem cells from human blood. *Blood* 113:5476–5479.
7. Abyzov A, J Mariani, D Palejev, Y Zhang, MS Haney, L Tomasini, AF Ferrandino, LA Rosenberg Belmaker, A Szekely, et al. (2012). Somatic copy number mosaicism in human skin revealed by induced pluripotent stem cells. *Nature* 492:438–442.
8. Buganim Y, DA Faddah and R Jaenisch. (2013). Mechanisms and models of somatic cell reprogramming. *Nat Rev Genet* 14:427–439.
9. Hansson J, MR Rafiee, S Reiland, JM Polo, J Gehring, S Okawa, W Huber, K Hochedlinger and J Krijgsvel. (2012). Highly coordinated proteome dynamics during reprogramming of somatic cells to pluripotency. *Cell Rep* 2:1579–1592.
10. Carey BW, S Markoulaki, C Beard, J Hanna and R Jaenisch. (2010). Single-gene transgenic mouse strains for reprogramming adult somatic cells. *Nat Methods* 7:56–59.
11. Eminli S, A Foudi, M Stadtfeld, N Maherali, T Ahfeldt, G Mostoslavsky, H Hock and K Hochedlinger. (2009). Differentiation stage determines potential of hematopoietic cells for reprogramming into induced pluripotent stem cells. *Nat Genet* 41:968–976.
12. Kang L, J Wang, Y Zhang, Z Kou and S Gao. (2009). iPS cells can support full-term development of tetraploid blastocyst-complemented embryos. *Cell Stem Cell* 5:135–138.
13. Tang F, C Barbacioru, E Nordman, B Li, N Xu, VI Bashkurov, K Lao and MA Surani. (2010). RNA-Seq analysis to capture the transcriptome landscape of a single cell. *Nat Protoc* 5:516–535.
14. Trapnell C, L Pachter and SL Salzberg. (2009). TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* 25:1105–1111.
15. Trapnell C, A Roberts, L Goff, G Pertea, D Kim, DR Kelley, H Pimentel, SL Salzberg, JL Rinn and L Pachter. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat Protoc* 7:562–578.
16. Wang L, Z Feng, X Wang, X Wang and X Zhang. (2010). DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics* 26:136–138.
17. Brambrink T, R Foreman, GG Welstead, CJ Lengner, M Wernig, H Suh and R Jaenisch. (2008). Sequential expression of pluripotency markers during direct reprogramming of mouse somatic cells. *Cell Stem Cell* 2:151–159.
18. Kou Z, L Kang, Y Yuan, Y Tao, Y Zhang, T Wu, J He, J Wang, Z Liu and S Gao. (2010). Mice cloned from induced pluripotent stem cells (iPSCs). *Biol Reprod* 83:238–243.
19. Osawa M, K Hanada, H Hamada and H Nakauchi. (1996). Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* 273:242–245.
20. Purton LE and DT Scadden. (2007). Limiting factors in murine hematopoietic stem cell assays. *Cell Stem Cell* 1:263–270.
21. Kiel MJ, OH Yilmaz, T Iwashita, OH Yilmaz, C Terhorst and SJ Morrison. (2005). SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* 121:1109–1121.
22. Marson A, R Foreman, B Chevalier, S Bilodeau, M Kahn, RA Young and R Jaenisch. (2008). Wnt signaling promotes reprogramming of somatic cells to pluripotency. *Cell Stem Cell* 3:132–135.
23. Samavarchi-Tehrani P, A Golipour, L David, HK Sung, TA Beyer, A Datti, K Woltjen, A Nagy and JL Wrana. (2010). Functional genomics reveals a BMP-driven mesenchymal-to-epithelial transition in the initiation of somatic cell reprogramming. *Cell Stem Cell* 7:64–77.
24. Li R, J Liang, S Ni, T Zhou, X Qing, H Li, W He, J Chen, F Li, et al. (2010). A mesenchymal-to-epithelial transition initiates and is required for the nuclear reprogramming of mouse fibroblasts. *Cell Stem Cell* 7:51–63.
25. Ichida JK, J Blanchard, K Lam, EY Son, JE Chung, D Egli, KM Loh, AC Carter, FP Di Giorgio, et al. (2009). A small-molecule inhibitor of tgfbeta signaling replaces sox2 in reprogramming by inducing nanog. *Cell Stem Cell* 5:491–503.
26. Maherali N and K Hochedlinger. (2009). Tgfbeta signal inhibition cooperates in the induction of iPSCs and replaces Sox2 and cMyc. *Curr Biol* 19:1718–1723.
27. Miettinen PJ, R Ebner, AR Lopez and R Derynck. (1994). TGF-beta induced transdifferentiation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors. *J Cell Biol* 127:2021–2036.
28. Chang G, S Gao, X Hou, Z Xu, Y Liu, L Kang, Y Tao, W Liu, B Huang, et al. (2014). High-throughput sequencing reveals the disruption of methylation of imprinted gene in induced pluripotent stem cells. *Cell Res* 24:293–306.

29. Guo S, X Zi, VP Schulz, J Cheng, M Zhong, SH Koochaki, CM Megyola, X Pan, K Heydari, et al. (2014). Non-stochastic reprogramming from a privileged somatic cell state. *Cell* 156:649–662.
30. Li W, H Zhou, R Abujarour, S Zhu, J Young Joo, T Lin, E Hao, HR Scholer, A Hayek and S Ding. (2009). Generation of human-induced pluripotent stem cells in the absence of exogenous Sox2. *Stem Cells* 27:2992–3000.
31. Ho R, B Papp, JA Hoffman, BJ Merrill and K Plath. (2013). Stage-specific regulation of reprogramming to induced pluripotent stem cells by wnt signaling and T cell factor proteins. *Cell Rep* 3:2113–2126.
32. Polo JM and K Hochedlinger. (2010). When fibroblasts MET iPSCs. *Cell Stem Cell* 7:5–6.

Address correspondence to:

Prof. Lan Kang
Institute of Cancer Stem Cell (ICSC)
Dalian Medical University
Dalian 116044
People's Republic of China

E-mail: kanglan@dlmedu.edu.cn

Prof. Shaorong Gao
School of Life Sciences and Technology
Tongji University
Shanghai 200092
People's Republic of China

E-mail: gaoshaorong@tongji.edu.cn

Prof. Qian-Fei Wang
Key Laboratory of Genomic and Precision Medicine
Beijing Institute of Genomics
Chinese Academy of Sciences
Beijing 100029
People's Republic of China

E-mail: wangqf@big.ac.cn

Received for publication September 2, 2014

Accepted after revision February 13, 2015

Prepublished on Liebert Instant Online March 9, 2015