Cell Stem Cell
Review

Direct Lineage Reprogramming: Strategies, Mechanisms, and Applications

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The direct lineage reprogramming of one specialized cell type into another using defined factors has fundamentally re-shaped traditional concepts regarding the epigenetic stability of differentiated cells. With the rapid increase in cell types generated through direct conversion in recent years, this strategy has become a promising approach for producing functional cells. Here, we review recent advances in lineage reprogramming, including the identification of novel reprogramming factors, underlying molecular mechanisms, strategies for generating functionally mature cells, and assays for characterizing induced cells. We also discuss progress toward the application of lineage reprogramming and the major future challenges for this strategy.

Introduction

The generation of desired functional cell types is a long-standing major goal of regenerative medicine, which holds great promise for biomedical applications. Although conventional strategies, particularly the directed differentiation of pluripotent stem cells, have been extensively studied for this purpose and have progressed rapidly in recent years (Murry and Keller, 2008; Tabar and Studer, 2014), there has also been great interest in the development of alternative strategies for obtaining functional cells. In principle, lineage reprogramming, which can be defined as the direct induction of functional cell types from one lineage to another lineage without passing through an intermediate pluripotent stage (Graf and Enver, 2009), could become an alternative way to produce the desired cell types. The feasibility of this strategy was first demonstrated by the conversion of fibroblasts into myoblasts by overexpressing *Myod*, which led to the conceptual breakthrough that a single cell-type-specific transcription factor can change cell fate (Davis et al., 1987). Subsequent studies showed that transcription factor-driven cell fate conversion could be achieved between related lineages such as different cell types within the blood, endoderm, and nervous system (Heins et al., 2002; Kulessa et al., 1995; Shen et al., 2000; Xie et al., 2004).

The advent of induced pluripotent stem cells (iPSCs), which demonstrates that a combination of cell-type-specific transcription factors can change cell fates between developmentally distant cell types, has reignited the field of lineage reprogramming (Takahashi and Yamanaka, 2006). The use of transcription factor combinations was quickly applied to the in vivo generation of pancreatic insulin-producing cells that alleviated hyperglycemia in diabetic mouse models (Zhou et al., 2008). Subsequently, the induction of neuronal cells by transcription factors proved that lineage conversion could be conducted across distant germ layers (Vierbuchen et al., 2010). Since then, lineage reprogramming using transcription factor combinations has also been used to induce several other cell types, such as cardiomyocytes and hepatocytes (Huang et al., 2011; Ieda et al., 2010; Sekiya and Suzuki, 2011). Inspiringly, this field has progressed rapidly in recent years, which has resulted not only in the identification of novel reprogramming factors but also the development of new strategies to improve the functional maturation of converted cells. As a result, the number of different cell types derived from lineage reprogramming in both mice and humans has substantially increased (Figure 1; Tables 1 and 2). Based on these recent advances, lineage reprogramming has become a widely used and promising approach for obtaining functional cell types. In this review, we discuss the major aspects of the recent progress in direct lineage reprogramming and provide perspectives for future challenges in the field.

Beyond Lineage-Specific Transcription Factor Overexpression: Novel Factors Involved in Lineage Reprogramming

Although the enforced expression of transcription factors has been extensively used to induce lineage conversion in multiple studies (Tables 1 and 2), there is still great interest in the identification of other factors capable of inducing lineage conversion. Notably, recent findings have indicated that alternative factors, including epigenetic regulators, microRNAs (miRNAs), and small molecules, have been implicated in lineage reprogramming (Figure 2). Importantly, the understanding of the molecular mechanisms underlying lineage reprogramming could potentially benefit from these studies. Furthermore, these novel factors may represent alternative ways of achieving cell fate conversion in the future.

Epigenetic Regulators. Fundamentally, lineage reprogramming involves the transition between different epigenetic states. In addition, during lineage reprogramming, exogenous factors must interact with or act upon epigenetic regulators to properly reactivate the epigenetically repressed state of the target cell-type-specific master genes. Therefore, it is reasonable that

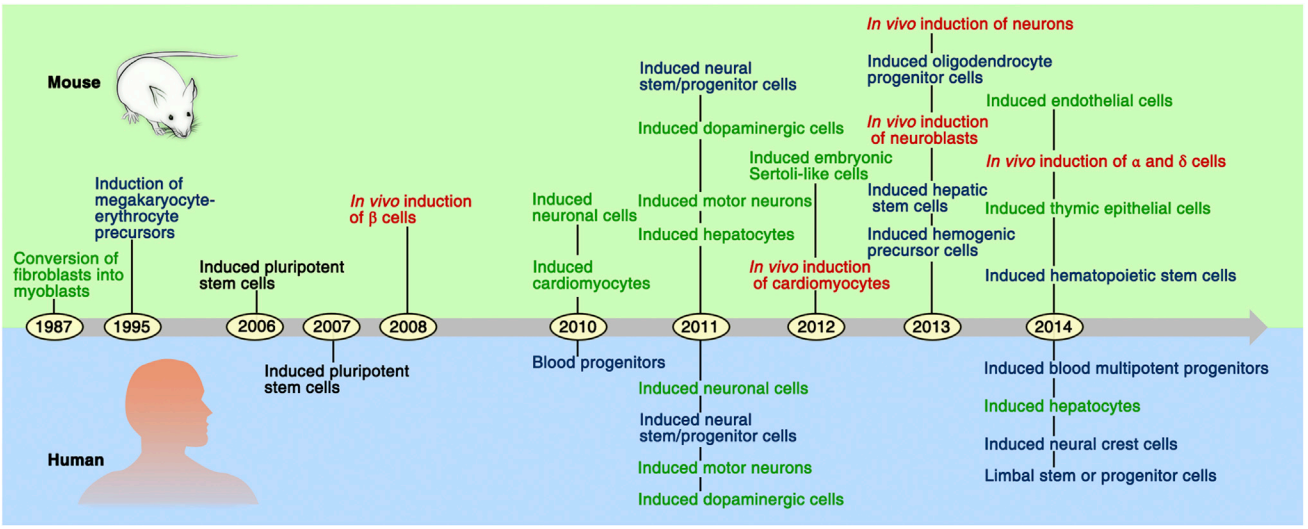

Figure 1. Historical View of the Development of Lineage Reprogramming

Selected advances in the development of lineage reprogramming are highlighted in different colors. Green, blue, and red indicate the induction of terminally differentiated cell types, stem cells or progenitors/precursors, and in vivo lineage reprogramming, respectively. Texts above the timeline indicate studies in mice, and texts below the timeline indicate studies in humans.

chromatin modifiers may participate in this process. In support of this notion, it has been reported that non-cardiac mesoderm in mouse embryos could be transdifferentiated into cardiomyocytes by the ectopic expression of the cardiac transcription factors *Gata4* and *Tbx5* in combination with the cardiac-specific subunit of BAF chromatin remodeling complexes, which are referred to as *Baf60c*. Mechanistically, the essential role of *Baf60c* during this conversion can be explained, in part, by permitting the binding of Gata4 to cardiac genes (Takeuchi and Bruneau, 2009).

Such studies illustrate that chromatin remodelers can facilitate lineage conversion through coordination with exogenous transcription factors; however, epigenetic barriers mediated by chromatin modifiers can also hinder lineage reprogramming. Epigenetic barriers during iPSC reprogramming have been extensively characterized (Buganim et al., 2013; Theunissen and Jaenisch, 2014), and some of the same barriers may also play a role in lineage reprogramming. For example, the inhibition or removal of chromatin modifiers such as histone deacetylases and polycomb repressor complex 2 (PRC2) can facilitate the conversion of germ cells to neurons driven by neuron-specific transcription factors in *Caenorhabditis elegans* (Patel et al., 2012; Tursun et al., 2011). Together, these findings indicate a complicated interaction between epigenetic regulators and extrinsic lineage-specific factors during lineage conversion.

The importance of epigenetic regulators during lineage reprogramming is further highlighted by the interesting fact that lineage conversion can occur through the manipulation of epigenetic regulators alone. In mice, a DNA methyltransferase *Dnmt1* deficiency in pancreatic β cells leads to their conversion into α cells (Dhawan et al., 2011). Further analysis indicated that *Arx*, a master gene responsible for the maintenance of α cell identity, was re-activated in β cells upon *Dnmt1* mutation (Courtney et al., 2013). Therefore, the disruption of DNA methylation may re-activate certain lineage-specific master genes that are sufficient to direct cell fates. In addition to the disruption of DNA methylation, the manipulation of histone-modifying enzymes may affect the lineage conversion process, because these histone modifications also correspond to transcriptionally active or silent chromatin states (Jenuwein and Allis, 2001). In *Caenorhabditis elegans*, it has recently been reported that the histone H3K27me2/3 demethylase Jmjd3.1 and the H3K4 methyltransferase Set1 complex can sequentially determine the transdifferentiation of hindgut cells into motor neurons (Zuryn et al., 2014).

Several important questions have emerged from studies that manipulate epigenetic regulators to induce lineage reprogramming. First, many of these studies have relied upon analyzing marker gene expression rather than functional output to characterize the identity of converted cells. Therefore, it is still unclear if functionally mature cells can be obtained using epigenetic regulators. Second, most of these conversions were conducted in vivo, and whether epigenetic regulators can function like transcription factors that induce lineage conversion in vitro remains unclear. Finally, questions regarding the mechanisms and specificity of action remain unanswered. In general, current studies support that two types of epigenetic regulators are involved in lineage reprogramming: one expressed specifically in certain lineages and the other expressed more broadly in different cell types. Although several studies have shown that lineage-specific epigenetic regulators may exert their function by specifically modulating the epigenetic state of lineage-specific genes (Takeuchi and Bruneau, 2009), it remains largely unknown how the manipulation of universal epigenetic regulators activates the core gene regulatory network (GRN), especially transcription factors whose expression is specific to the target cell type. One possibility is that they may drive the lineage conversion in a context-dependent manner; that is, their function relies on signaling pathways and transcription factors already present in the starting population. Understanding these interactions may

better facilitate the identification of proper epigenetic regulators promoting lineage reprogramming.

miRNAs. Emerging evidence indicates that miRNAs are important players in lineage reprogramming. For induced neuronal (iN) cell induction, the overexpression of the neuronal-specific miRNAs *miR-9/9** and *miR-124* alone in human fibroblasts can induce neuron-like cells that express the neuronal marker MAP2; however, the addition of transcription factors is required for functional human iN cell formation (Yoo et al., 2011). In another study, the overexpression of *miR-124* also promoted human iN cell induction mediated via *BRN2* and *MYT1L* overexpression (Ambasudhan et al., 2011). In addition to iN cell induction, miRNA-driven cardiac lineage conversion can be induced in vitro and in vivo via the use of cardiac enriched miRNAs *miR-1*, *miR-133*, *miR-208*, and *miR-499* alone (Jayawardena et al., 2012). The substantial role of miRNAs in lineage reprogramming has been further revealed by the surprising fact that the induction of functional murine iN cells can be achieved via the inhibition of the miRNA regulator PTB alone (Xue et al., 2013). PTB blocks miRNA-mediated activity of the REST complex, thus PTB inhibition promotes iN cell induction by allowing de-repression of multiple miRNA-regulated neuronal genes.

In general, it seems that miRNA-induced lineage conversion is currently still not as efficient as transcription-factor-mediated lineage conversion. For instance, the enforced overexpression of miRNAs alone only induces neural marker gene expression in human non-neural cells (Ambasudhan et al., 2011; Yoo et al., 2011), which was in contrast to the relatively complete induction of functional human neuronal cells by transcription factors (Pang et al., 2011). While PTB inhibition leads to functional neuronal induction in mice (Xue et al., 2013), it is possible that mechanisms other than miRNA regulation may also be involved in neuronal induction triggered by PTB inhibition. For example, inhibition of the REST complex, which was thought to be an important player in the PTB-regulated miRNA program in neuronal induction, cannot fully recapitulate the effects of PTB inhibition (Xue et al., 2013). It is possible that alternative splicing is also involved, because PTB is a well-known splicing regulator (Oberstrass et al., 2005). While the reported miRNA-mediated lineage conversions need to be further confirmed, it is also important to determine whether the overexpression of exogenous miRNAs alone is sufficient to induce functional converted cells either in vitro or in vivo.

Although current studies provide insight into miRNA-mediated cell fate conversion, the molecular mechanisms of this process remain elusive. Considering that miRNAs typically regulate their targets through repression (Bartel, 2009), how miRNAs activate master gene expression for a target cell type is an appealing question. One possibility is that the overexpression of lineage-specific miRNAs may downregulate master regulators that are expressed in the initial cells or the transition cells, which may lead to the disruption of balance among master regulators of different lineages. For example, it has been reported that neuronal-specific *miR-124a* decreases the levels of hundreds of non-neuronal transcripts even when expressed in non-neural cells (Lim et al., 2005). This inhibition may also inhibit non-neuronal master genes and shift the balance toward the expression of neuronal master genes, thus pushing the cells toward neural conversion. Another possibility is that miRNAs may downregulate the expression of certain epigenetic regulators and promote the global epigenetic changes in certain in vitro or in vivo contexts. For example, *miR-124* directly regulates the expression of *Ezh2*, a histone H3 Lys-27 histone methyltransferase, which facilitates the expression of neural-specific *Ezh2* target genes (Neo et al., 2014). While these possibilities still need to be confirmed by experimental evidence, studies revealing how miRNA-induced lineage conversion occurs could deepen the broader understanding of mechanisms underlying lineage reprogramming.

Small Molecules. The use of genetic manipulation in lineage reprogramming raises safety concerns for its clinical applications, and one of the most promising potential solutions is the induction of this conversion by small molecules. Small molecules have several prominent advantages over traditional methods for regulating cell fate: they can be cell permeable; more cost-effective; and more easily synthesized, preserved, and standardized. More importantly, the effects of small molecules can be fine-tuned by varying their concentrations and combinations, thus providing a higher degree of temporal and spatial control over protein function (Li et al., 2013b). Small molecules have been widely used in iPSC reprogramming (Li et al., 2013b). Remarkably, iPSCs could be generated using the combinations of small molecules alone (Hou et al., 2013). Recently, several small molecules have been reported to promote the efficiency of neural conversion, to reduce the requirement for exogenous factors, or to directly induce cell fate conversion (Cheng et al., 2014; Kim et al., 2014; Ladewig et al., 2012; Liu et al., 2013; Sayed et al., 2015; Zhu et al., 2014a). However, identifying small molecules that completely replace exogenous transcription factors during lineage reprogramming remains a major challenge for most lineages.

Natural examples of dedifferentiation or transdifferentiation processes have been observed in several vertebrate species (Jopling et al., 2011) and can provide insight on identifying small molecules that could modulate these pathways. In principle, the transition between different cellular states is the output of interactions among multiple factors such as signal pathways, transcription factors, and epigenetic regulators, and signal pathways and epigenetic regulators are involved in these natural lineage conversions. For instance, Wnt signaling has been shown to play a deterministic role during lens regeneration in adult newts (Hayashi et al., 2006), which involves the conversion of pigmented epithelial cells to lens cells. Epigenetic modifiers such as histone deacetylases are also reported to be upregulated during this process (Maki et al., 2010). These natural examples suggest the possibility of manipulating signaling pathways and epigenetic regulators to induce lineage conversion, and this could theoretically be achieved by small molecules that modulate these pathways and factors, which is indeed the case for chemical iPSC induction (Hou et al., 2013). Furthermore, for successful chemically induced lineage conversion, careful titration of the dosage and duration of different small molecules may be critical. For instance, to activate endogenous *Oct4* activity, the SAH hydrolase inhibitor 3-deazaneplanocin A (DZNep) could only be added during the late stage of chemical reprogramming (Hou et al., 2013).

Another intriguing question is how small molecules could replace the function of exogenous transcription factors during

Table 1. Summary of Lineage Reprogramming in Mice since the Discovery of iPSCs

In Vitro or In Vivo	Initial Cell Population	Target Cell Type	Reprogramming Factors	Reference
In vitro	Fibroblasts	Adipocytes (brown fat cells)	PRDM16, CEBPβ	Kajimura et al., 2009
In vitro	Fibroblasts	Astrocytes	Nfia, Nfib, Sox9	Caiazzo et al., 2014
Both	Fibroblasts	Cardiomyocytes	Gata4, Hand2, Mef2c, Tbx5	Song et al., 2012
Both	Fibroblasts	Cardiomyocytes	miR-1, miR-133, miR-208, miR-499, JAK inhibitor I	Jayawardena et al., 2012
In vitro	Fibroblasts	Cardiomyocytes	Gata4, Mef2c, Tbx5	Ieda et al., 2010
In vitro	Fibroblasts	Cardiomyocytes	Hand2, Nkx2.5, Gata4, Mef2c, Tbx5	Addis et al., 2013
In vitro	Fibroblasts	Cardiomyocytes	Mef2c, Myocd, Tbx5	Protze et al., 2012
In vitro	Fibroblasts	Cardiomyocytes	MYOCD, SRF, Mesp1, GATA4, TBX5, MEF2C, SMARCD3	Christoforou et al., 2013
In vivo	Fibroblasts	Cardiomyocytes	Gata4, Mef2c, Tbx5	Qian et al., 2012
In vivo	Fibroblasts	Cardiomyocytes	Gata4, Mef2c, Tbx5	Inagawa et al., 2012
In vitro	Fibroblasts	Chondrocytes	Sox9, Klf4, c-Myc	Hiramatsu et al., 2011
In vivo	Rod photoreceptors	Cone-like cells	Nrl ablation	Montana et al., 2013
In vitro	Fibroblasts	Endothelial cells	Foxo1, Er71, Klf2, Tal1, Lmo2	Han et al., 2014
In vitro	Fibroblasts	Hemogenic endothelial-like precursor cells	Gata2, Gfi1b, cFos, Etv6	Pereira et al., 2013
In vitro	Fibroblasts	Haematopoietic progenitor cells	ERG, GATA2, LMO2, RUNX1c, SCL, p53(−/−)	Batta et al., 2014
In vivo	Committed lymphoid, myeloid progenitors, and myeloid effector cells	Haematopoietic stem cells	Run1t1, Hlf, Lmo2, Prdm5, Pbx1, Zfp37, Mycn, Meis1	Riddell et al., 2014
In vitro	Fibroblasts	Hepatic stem cells	Hnf1β, Foxa3	Yu et al., 2013
In vitro	Fibroblasts	Hepatocytes	Gata4, Hnf1α, Foxa3, P19ARF knockdown	Huang et al., 2011
In vitro	Fibroblasts	Hepatocytes	Hnf4α plus Foxa1, Foxa2 or Foxa3	Sekiya and Suzuki, 2011
In vivo	Layer II/III callosal projection neurons	Layer-V/VI corticofugal projection neurons	Fezf2	Rouaux and Arlotta, 2013
In vitro	Fibroblasts	Macrophages	PU.1 plus CEBPα or CEBPβ	Feng et al., 2008
In vitro	B cells	Macrophages	CEBPα	Bussmann et al., 2009
In vitro	Fibroblasts	Melanocytes	MITF, SOX10, PAX3	Yang et al., 2014
In vitro	Neural stem cells	Monocytes	PU.1	Forsberg et al., 2010
In vivo	T cells	Natural killer-like cells	Bcl11b deletion	Li et al., 2010
In vitro	Fibroblasts	Neural precursor cells	Brn2, Sox2, FoxG1	Lujan et al., 2012
In vitro	Fibroblasts	Neural progenitor cells	VPA, CHIR99021, RepSox (616452) under hypoxia	Cheng et al., 2014
In vitro	Fibroblasts	Neural stem cells	Brn4, Sox2, Klf4, c-Myc, E47	Han et al., 2012
In vitro	Fibroblasts	Neural stem cells	Sox2, Klf4,c-Myc, Oct4 (limiting activity at initial stage)	Thier et al., 2012
In vitro	Fibroblasts	Neural stem cells	Sox2	Ring et al., 2012
In vitro	Sertoli cells	Neural stem cells	Ascl1, Ngn2, Hes1, Id1, Pax6, Brn2, Sox2, c-Myc, Klf4	Sheng et al., 2012b
In vivo	Astrocytes	Neuroblasts	Sox2	Niu et al., 2013
in vitro	Hepatocytes	Neurons	Ascl1, Brn2, Myt1l	Marro et al., 2011
In vitro	Fibroblasts	Neurons	PTB repression	Xue et al., 2013
In vitro	Fibroblasts	Neurons	ASCL1	Chanda et al., 2014
In vivo	Astrocytes	Neurons	Ascl1, Brn2, Myt1l	Torper et al., 2013
In vivo	Astrocytes	Neurons (glutamatergic)	NeuroD1	Guo et al., 2014
In vitro	Fibroblasts	Neurons (dopaminergic)	Ascl1, Pitx3, Lmx1a, Nurr1, Foxa2, EN1	Kim et al., 2011b
In vitro	Fibroblasts	Neurons (dopaminergic)	Ascl1, Lmx1a, Nurr1	Caiazzo et al., 2011

(*Continued on next page*)

Table 1. *Continued*

In Vitro or In Vivo	Initial Cell Population	Target Cell Type	Reprogramming Factors	Reference
In vitro	Fibroblasts	Neurons (dopaminergic)	Lmx1a, Foxa2, Ascl1, Brn2 or Lmx1b, Otx2, Nurr1, Ascl1, Brn2	Sheng et al., 2012a
In vitro	Astrocytes	Neurons (GABAergic)	Ascl1, Dlx2	Heinrich et al., 2010
In vivo	NG2 cells	Neurons (glutamatergic and GABAergic)	NeuroD1	Guo et al., 2014
In vitro	Fibroblasts	Neurons (glutamatergic)	Ascl1, Brn2, Myt1l	Vierbuchen et al., 2010
In vitro	Astrocytes	Neurons (glutamatergic)	Ngn2	Heinrich et al., 2010
In vitro	Fibroblasts	Neurons (motor)	Brn2, Ascl1, Myt1l, Lhx3, Hb9, Isl1, Ngn2	Son et al., 2011
In vitro	Fibroblasts	Oigodendrocyte progenitor cells	Olig1, Olig2, Nkx2.2, Nkx6.2, Sox10, ST18, Gm98, Myt1	Najm et al., 2013
In vitro	Fibroblasts	Oligodendrocyte progenitor cells	Sox10, Olig2, Zfp536	Yang et al., 2013
In vivo	Hepatic progenitor cells	Pancreatic islet cells	Ngn3	Yechoor et al., 2009
In vivo	Pancreatic β cells	Pancreatic α cells	Dnmt1 deficiency	Dhawan et al., 2011
In vivo	Pancreatic acinar cells	Pancreatic α cells	Ngn3, MafA	Li et al., 2014b
In vivo	Pancreatic acinar cells	Pancreatic β cells	Ngn3, Pdx1, MafA	Zhou et al., 2008
In vivo	Pancreatic acinar cells	Pancreatic δ cells	Ngn3	Li et al., 2014b
In vitro	Fibroblasts	Sertoli cells	Nr5a1, Wt1, Dmrt1, Gata4, Sox9	Buganim et al., 2012
In vivo	Granulosa cells	Sertoli cells	Deletion of Foxl2	Uhlenhaut et al., 2009
In vitro	Fibroblasts	Thymic epithelial cells	Foxn1	Bredenkamp et al., 2014

the chemical conversion process. Although the difference of global gene expression between different cell types may involve thousands of genes, the core GRN that determines one specific cell type may only be comprised of several master genes. For example, a recent study indicated that the essential transcription factor program of mouse naive pluripotent stem cells involves 16 interactions, 12 components, and three inputs (Dunn et al., 2014). Importantly, because there are multiple interactions between factors of the core GRN, activation of certain lineage-specific master genes may trigger feedback that eventually activates the whole core GRN for a specific lineage. Accordingly, activation of a few master genes for a specific lineage may prime cell fate conversion toward that lineage, and functional induced cells could be generated with proper culture conditions. Remarkably, small molecules could achieve activation of certain factors such as transcription factors. For example, small molecules could activate the expression of two pluripotency genes *Sall4* and *Sox2*, during the early phase of chemical reprogramming (Hou et al., 2013). Another example is the identification of a small molecule that activates the expression of a critical pancreatic regulator *PDX1* in human ductal carcinoma cell lines and primary human islets (Yuan et al., 2013). Considering that an important step during transcription-factor-mediated lineage reprogramming is the activation of the core GRN of the target cell type, small-molecule-mediated activation of factors comprising the core GRN may partially explain why small molecules could replace the role of exogenous transcription factors during lineage conversion. On the other hand, the exact mechanism of small-molecule-mediated activation of factors comprising the core regulatory network for a specific cell type remains elusive and requires future studies.

Pluripotency Factors for Indirect Lineage Reprogramming. Recently, there has also been an increasing interest in the development of an indirect lineage reprogramming strategy using pluripotency factors. Several cell types have been induced using this strategy, including cardiomyocytes (Efe et al., 2011), neural stem cells or progenitors (Corti et al., 2012; Kim et al., 2011a; Thier et al., 2012; Wang et al., 2013), pancreatic lineages (Li et al., 2014a), angioblast-like progenitor cells (Kurian et al., 2013), endothelial cells (Li et al., 2013a), and hepatocytes (Zhu et al., 2014b) in either mouse or human cells. It has been proposed that pluripotency-factor-driven lineage conversion is dependent on the presence of an epigenetically unstable/plastic population at early and intermediate stages during reprogramming, which may be directed to an alternative cell fate other than iPSCs under proper signaling environments (Ma et al., 2013).

During the early phase of reprogramming, exogenous pluripotency factors like *Oct4* can act as pioneer factors that bind to the regulatory regions of many genes throughout the genome (Buganim et al., 2013). Accumulating evidence has shown that pluripotency factors can interact with different epigenetic modulators such as NuRD, BAF, or the PRC complex (Huang and Wang, 2014). Therefore, it is possible that the binding of exogenous pioneer factors could recruit these epigenetic modulators and facilitate the re-activation of epigenetically repressed lineage-specific genes. This effect may be further strengthened by the proper addition of compounds that regulate key signal pathways or epigenetic modulators. On the other hand, exogenous pluripotency factors themselves may also act as lineage specifiers during the early stages of the reprogramming process, because it has been recently suggested that exogenous pluripotency factors may act as lineage specifiers during reprogramming to pluripotency (Shu et al., 2013). Furthermore, the overexpression of

Table 2. Summary of Lineage Reprogramming in Humans since the Discovery of iPSCs

Initial Cell Population	Target Cell Type	Reprogramming Factors	Reference
Fibroblasts	Adipocytes (brown fat cells)	PRDM16, CEBPβ	Kajimura et al., 2009
Endothelial cells	Haematopoietic multipotent progenitor cells	FOSB, GFI1, RUNX1, SPI1	Sandler et al., 2014
Fibroblasts	Haematopoietic progenitors	OCT4	Szabo et al., 2010
Fibroblasts	Cardiac progenitors	ETS2, MESP1	Islas et al., 2012
Fibroblasts	Cardiomyocytes	GATA4, HAND2, MYOCD, TBX5, miR-1, miR-133	Nam et al., 2013
Fibroblasts	Cardiomyocytes	GATA4, MEF2C, TBX5, MYOCD, MESP1	Wada et al., 2013
Fibroblasts	Cardiomyocytes	GATA4, MEF2C, TBX5, ESRRG, MESP1, Myocardin, ZFPM2	Fu et al., 2013
Amniotic cells	Endothelial Cells	ETV2, FLI1, ERG1 (and TGFβ inhibition)	Ginsberg et al., 2012
Fibroblasts	Hepatocytes	HNF1A, HNF4A, HNF6, CEBPA, ATF5, PROX1, p53-siRNA, C-MYC	Du et al., 2014
Fibroblasts	Hepatocytes	HNF1A, HNF4A, FOXA3, SV40 large T antigen	Huang et al., 2014
Skin epithelial stem cells	Limbal stem or progenitor cells	PAX6	Ouyang et al., 2014
Fibroblasts	Melanocytes	MITF,SOX10,PAX3	Yang et al., 2014
Fibroblasts	Monocyte-like progenitor cells	SOX2, miR-125b	Pulecio et al., 2014
Proximal tubule (HK2) cell line	Nephron progenitors	SIX1, SIX2, OSR1, EYA1, HOXA11, SNAI2	Hendry et al., 2013
Fibroblasts	Neural crest cells	SOX10	Kim et al., 2014
Fibroblasts	Neural stem cells	SOX2	Ring et al., 2012
Fibroblasts	Neurons	ASCL1, NGN2, CHIR99021, SB431542	Ladewig et al., 2012
Fibroblasts	Neurons (glutamatergic)	NGN2, Forskolin, Dorsomorphin	Liu et al., 2013
Fibroblasts	Neurons	ASCL1	Chanda et al., 2014
Pericyte-derived cells	Neurons	SOX2, MASH1(also named ASCL1)	Karow et al., 2012
Fibroblasts	Neurons (dopaminergic)	ASCL1, BRN2, MYT1L, LMX1A, FOXA2	Pfisterer et al., 2011
Fibroblasts	Neurons (dopaminergic)	ASCL1, LMX1A, NURRL	Caiazzo et al., 2011
Fibroblasts	Neurons (dopaminergic)	MASH1, NGN2, SOX2, NURR1, PITX3	Liu et al., 2012
Fibroblasts	Neurons (glutamatergic)	ASCL1, BRN2, MYT1L, NEUROD1	Pang et al., 2011
Fibroblasts	Neurons (glutamatergic)	BRN2, MYT1L, miR-124	Ambasudhan et al., 2011
Fibroblasts	Neurons (glutamatergic, GABAergic)	ASCL1, MYT1L, NEUROD2, miR-9/9*, miR-124	Yoo et al., 2011
Fibroblasts	Neurons (motor)	BRN2, ASCL1, MYT1L, LHX3, HB9, ISL1, NGN2	Son et al., 2011
Fibroblasts	Retinal pigment epithelium-like cells	PAX6, RAX, CRX, MITF-A, OTX2, NRL, KLF4, C-MYC, Activin A or RA + Shh treatment at later stages	Zhang et al., 2013
Pancreatic exocrine cells	Pancreatic β-like cells	Activated MAPK and STAT3	Lemper et al., 2014

pluripotency factors in embryonic stem cells (ESCs) has been shown to induce differentiation (Loh and Lim, 2011). While the exact mechanisms of pluripotency-factor-driven lineage conversion still need further study, the potential presence of a transient pluripotent state during the conversion process remains a possibility. Although it has been shown that the expression of endogenous pluripotency genes such as *Nanog* was low or undetectable in these studies (Efe et al., 2011; Kim et al., 2011a; Li et al., 2014a; Zhu et al., 2014b), more rigorous assays, such as lineage tracing, are required to completely exclude this possibility.

Exploration of the Molecular Mechanisms of Lineage Reprogramming

To clarify the mechanism of lineage reprogramming, it is critical to define how exogenous factors drive the conversion process on the molecular level. Because the master genes of the target cell type are mostly epigenetically silenced in the initial cell population, an important question is how the regulatory network of the target cell type is reactivated in reprogrammed cells during conversion. Recently, significant insight has been gained from the mechanistic analysis of iN cell induction from murine fibroblasts, which have been driven by the enforced expression of *Brn2*, *Ascl1*, and *Myt1l* (BAM) (Wapinski et al., 2013). Wapinski et al. (2013) determined that the genomic binding patterns of *Ascl1*, a basic helix-loop-helix (bHLH) transcription factor, are similar between fibroblasts and neural progenitor cells. They further demonstrated that exogenous *Ascl1* binds to its authentic neuronal target genes in fibroblasts at the early phase of iN cell induction and facilitates the proper recruitment of other exogenous factors, such as *Brn2*, to their binding sites in later stages. Accordingly, *Ascl1* acts as an ''on-target pioneer factor'' during iN cell induction and can bind to its lineage-specific genomic

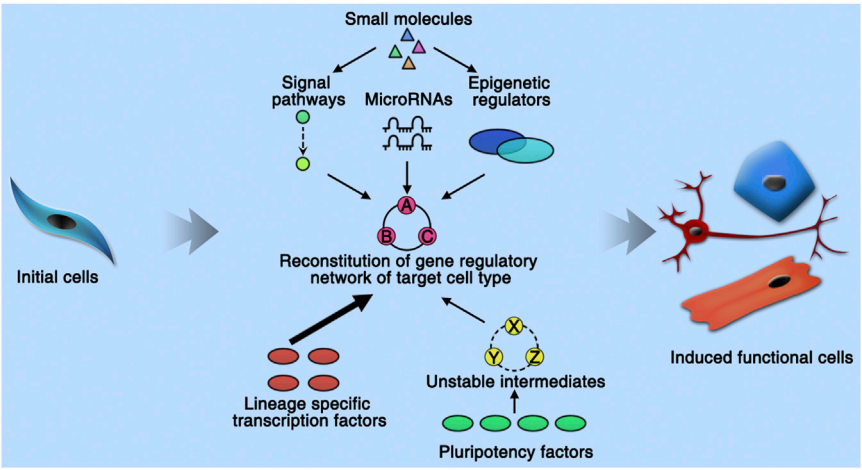

Figure 2. Factors Involved in Lineage Reprogramming

Reprogramming factors, including lineage-specific transcription factors, small molecules, epigenetic regulators, miRNAs, and pluripotency factors, are manipulated to direct cell fate toward the desired lineages. The ultimate downstream effects of these factors are the re-establishment of the GRN for the target cell type.

targets alone regardless of whether these targets are epigenetically activated or not (Zaret and Carroll, 2011). Therefore, Wapinski et al. (2013) proposed a hierarchical mechanism responsible for the BAM-factor-induced neuron conversion. Interestingly, the reprogramming factors responsible for iPSC induction also have pioneer properties (Soufi et al., 2012). In contrast to the pioneer factor *Ascl1*, which can bind to its neural targets in fibroblasts alone (Wapinski et al., 2013), the pluripotency factors *Oct4*, *Sox2*, and *Klf4* cooperatively function as pioneer factors; however, their initial binding regions in fibroblasts are largely different from the regions in pluripotent stem cells (Buganim et al., 2013). Thus, although one can hypothesize that, in general, pioneer factors play an important role during cell fate conversion, the exact mechanism of these factors in lineage conversion may be cell type dependent.

The use of factor combinations to induce lineage conversion raises an important question: do different expression levels of different exogenous factors affect the outcome of lineage reprogramming? Results from the mechanistic analysis of iPSC reprogramming have provided evidence that factor stoichiometry not only influences the reprogramming process but also the quality of iPSCs (Carey et al., 2011; Papapetrou et al., 2009; Tiemann et al., 2011; Yamaguchi et al., 2011). For example, high levels of exogenous Oct4 and Klf4 protein combined with low levels of Sox2 and c-Myc resulted in the generation of high-quality iPSCs with the ability of generating mice through tetraploid complementation (Carey et al., 2011). Accordingly, one can hypothesize that factor stoichiometry may also influence the conversion efficiency and functionality of converted cells in lineage reprogramming. Interestingly, one recent report has shown that higher levels of exogenous *Mef2c* with lower levels of *Gata4* and *Tbx5* significantly enhanced cardiac reprogramming efficiency (Wang et al., 2014), thus suggesting the important role of factor stoichiometry in lineage reprogramming.

The mechanistic analysis of the conversion between cell types could also benefit from other insights from iPSC reprogramming. For example, a "seesaw" model for reprogramming has recently been proposed by our group (Shu et al., 2013), in which a balance that is established using pluripotency factors and/or counteracting lineage specifiers can facilitate reprogramming to pluripotency. Intriguingly, this model also proposed that deviation from the balanced equilibrium for pluripotency directs cells to flow into divergent differentiated states. For example, the classical pluripotency factor *Oct4* has been proposed to be a mesendodermal lineage specifier that inhibits ectodermal potential under certain conditions in our model (Shu et al., 2013) and was previously shown to induce mesodermal differentiation when overexpressed in ESCs (Niwa et al., 2000). In lineage reprogramming, the overexpression of *OCT4* alone drove the conversion into mesodermal lineage hematopoietic cells (Szabo et al., 2010). In contrast, strictly limited *Oct4* expression at the initial phase of reprogramming combined with constitutive *Sox2*, *Klf4*, and *c-Myc* overexpression resulted in ectodermal neural lineage conversion (Thier et al., 2012). Interestingly, early studies on lineage switching within the blood system also demonstrated transcription factor cross-antagonism during these conversions (Heyworth et al., 2002; Nerlov and Graf, 1998). Collectively, these studies suggest that the balance between different lineage specifiers may play an important role in the direction of cell fate conversion.

Strategies for Improving the Functional Maturation of Converted Cells

The generation of functionally mature cells is fundamental to many regenerative medical applications such as disease modeling, drug development, and, particularly, cell therapy. In principle, lineage reprogramming can generate functional cells because this strategy can bypass the multiple steps of lineage specification during development and directly convert one lineage to another. However, the execution of this strategy for generating different types of fully functional mature cells, especially terminally differentiated cells, remains a major challenge. For instance, it has been recently demonstrated that in most studies, directly converted cells fail to silence the expression programs of the initial population, which strongly indicates that they possess an immature phenotype (Cahan et al., 2014). As subsequently discussed, the recent emergence of several novel strategies may lead to the development of a fundamental solution for this key issue in future studies.

Cell Fate Determination Factors May Not Be Sufficient for Inducing Fully Functional Maturation. In most cases, reprogramming factors are selected based on their importance in lineage specification during embryonic development. Therefore, we will refer to these factors as cell fate determination factors. However, it is unclear whether cell fate determination factors can also prime the entire functional maturation network during lineage reprogramming. Several studies suggest that this may not work, at least for the induction of certain cell types with multiple complex

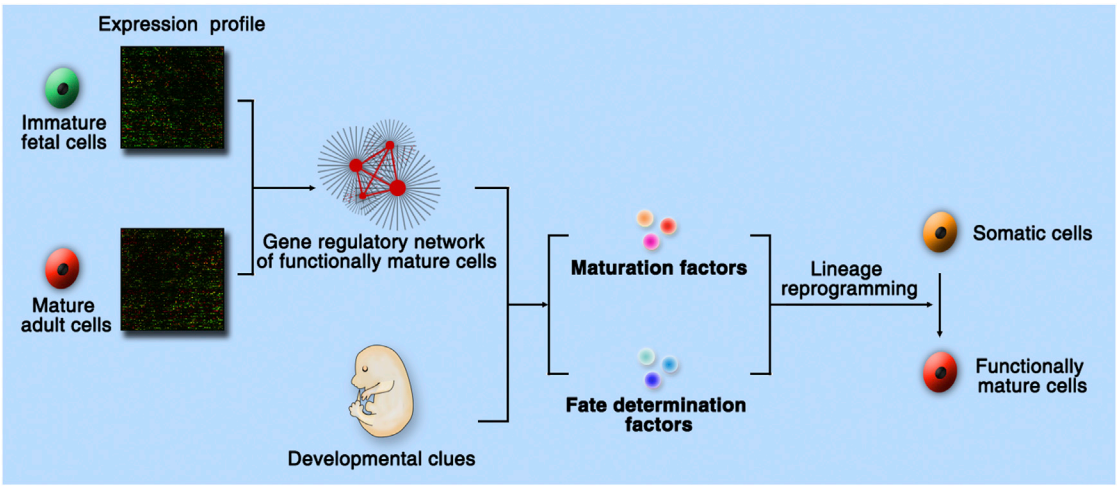

Figure 3. Emergence of a Novel Approach for the Generation of Functionally Mature Cells by Lineage Reprogramming
The generation of functionally mature cells by lineage reprogramming benefits from the combination of cell fate determination and maturation factors. In addition to developmental clues, both cell fate determination and maturation factors can be identified through the comparison of global gene expression profiles in immature fetal cells and mature adult cells. Further analysis of the GRN in these groups could facilitate the discovery of key cell fate determination and maturation factors responsible for cell fate conversion and functional maturation, respectively. Finally, the combination of cell fate determination and maturation factors can convert the original cells into the desired cell type with fully functional maturation.

functions. For example, hepatocytes perform hundreds of functions, including metabolic, synthetic, immunologic, and detoxification processes (Bhatia et al., 2014), and may be one of the most functionally complicated cell types. Mouse hepatocyte-like cells induced by hepatic fate determination transcription factors remained functionally immature, which was reflected by the incomplete hepatocyte differentiation and expression of certain hepatoblast markers (Huang et al., 2011; Sekiya and Suzuki, 2011). Furthermore, an unexpected intestinal gene expression program was detected in murine hepatocyte-like cells (Morris et al., 2014). Similarly, the combination of hepatic cell determination factors *HNF1A*, *HNF4A*, and *HNF6* could only induce hepatocyte-like cells that lacked key functional features of human hepatocytes (Du et al., 2014). Therefore, fate determination factors alone may not be sufficient to achieve functional maturation of converted cells.

Combination of Cell Fate Determination and Maturation Factors. Although several functional cell types, such as neuronal cells, can be induced by lineage reprogramming using cell fate determination factors alone (Vierbuchen et al., 2010), the functional maturation of other lineages, such as hepatocytes, may require additional factors during lineage conversion. How to identify such factors remains an important issue. In this regard, the induction of functional human hepatocytes may provide an example (Du et al., 2014). To identify additional factors that promote the functional maturation of hepatic-fate-determination-factor-induced hepatocyte-like cells, we compared the global gene expression patterns of induced cells, immature fetal hepatocytes, and freshly isolated primary human hepatocytes. This approach led to the identification of three mature hepatocyte-enriched transcription factors: *CEBPA*, *ATF5*, and *PROX1*, which we termed maturation factors. Intriguingly, the combination of these maturation factors with cell fate determination factors resulted in the induction of human induced hepatocytes (hiHeps), which functionally reconstituted the central network of drug metabolism (Du et al., 2014). Importantly, the activities of key drug metabolic enzymes, such as CYP3A4 in hiHeps, were also comparable to freshly isolated primary human hepatocytes. Moreover, these induced cells exhibited high levels of engraftment in vivo (repopulating up to 30% of the recipient livers) and secreted high levels of ALBUMIN comparable to that in primary human hepatocytes. Considering that these key functional properties of hepatocytes were not observed in hepatic-fate-determination-factor-induced cells, the inclusion of hepatic maturation factors in the factor combination plays a critical role in obtaining hepatic drug metabolic function by lineage reprogramming.

The strategy to generate functional hiHeps may be instructive for improvements in the function of converted cells from other lineages. Our findings suggest that fate determination and functional maturation may be governed by different master genes and are somewhat independent of each other. As a result, the ectopic expression of cell fate determination factors may not be sufficient to generate fully functional cells, and the addition of functional maturation factors are required to promote this process. Importantly, more recent studies have suggested that these maturation factors could be identified through the analysis of GRNs in adult functional target cell types (Morris et al., 2014). Thus, we suggest that the combination of cell fate determination and maturation factors may represent an effective approach to achieve functional maturation by lineage reprogramming, which may facilitate the induction of functionally mature cells of other lineages (Figure 3). Furthermore, this strategy may also facilitate the functional maturation of differentiated cells derived from pluripotent stem cells, because previous studies showed that the enforced expression of certain exogenous factors can promote the functional maturation of differentiated cells during the directed differentiation process (Ionta et al., 2015; Kaneto et al., 2005; Kyba et al., 2002; Martinat et al., 2006; Zhao et al., 2013).

Development of Functional Screening Assays. To identify the optimal combination of factors for the induction of desired cell

types, an effective screening assay is required. In general, the expression of lineage-specific markers is widely used as the indicator for successful cell fate conversion, which has led to the induction of a number of lineages such as neuronal cells and cardiomyocytes (Ieda et al., 2010; Vierbuchen et al., 2010). However, there is a potential problem in this marker-based screening approach, because the expression of lineage-specific markers may not be directly linked to the function of converted cells. Thus, factor combinations identified by this screening assay may not result in fully functional mature cells. Therefore, the development of novel screening assays directly based on cell function is in high demand in this field.

Recently, a significant breakthrough has been achieved toward the development of a functional screening assay for generating transplantable hematopoietic stem cell (HSC) by lineage reprogramming. Derrick Rossi and colleagues adopted an elegant in vivo functional screening strategy by transplanting cells transduced with HSC-enriched factors into lethally irradiated congenic mice (Riddell et al., 2014). This strategy took advantage of a unique feature of HSCs: that even one functional HSC is capable of reconstituting the entire spectrum of blood lineages in vivo. Indeed, all lineages of hematopoietic progeny were detected in the recipient mice, which led to the identification of 6 transcription factors *Hlf*, *Runx1t1*, *Pbx1*, *Lmo2*, *Zfp37*, and *Prdm5* that were required for the generation of induced HSCs (iHSCs). This study illustrates how functional screens can be applied to the induction of functionally competent cells. However, it is also notable that this in vivo functional screening assay cannot be simply applied to the generation of other lineages due to its reliance on this unique feature of HSCs. To develop effective functional screening assays for lineage reprogramming in other cell types, the development and integration of novel technologies may be beneficial, and these developed functional assays will likely need to be cell type specific.

Creation of a Niche for the Functional Maturation of Converted Cells. The maintenance of functional converted cells independent of the overexpression of exogenous factors is a significant issue for the successful generation of functional cells. For most cell types, such as HSCs, the maintenance of functionality using the culture medium alone is difficult because the essential factors or cellular signaling responsible for cell function maintenance are not fully understood. One potential solution for this dilemma is to co-culture the reprogrammed cells with supporting cells from the endogenous niche where the target cells are maintained. For instance, in an attempt to induce human multipotent progenitor cells (MPPs) from human umbilical vein endothelial cells (HUVECs), Sandler et al. co-cultured HSC factor-transduced HUVECs with engineered endothelial cells during conversion, which mimicked the vascular microenvironment that supports HSC specification during development (Sandler et al., 2014). The combination of this niche with a serum-free medium permitted the induction of hematopoietic-like colonies, which serially engrafted in vivo and further differentiated into mature blood cells including B cells. Collectively, these results suggest the critical roles of the culture environment for the induction and maintenance of functional converted cells, and the potential use of supporting cells from the endogenous niche in the promotion of cell functional maturation.

Compared with the use of supporting cells in vitro, perhaps a more effective way to promote cell functional maturation is to take advantage of the in vivo niche, which has been adopted to promote the further differentiation and functional maturation of pancreatic progenitors derived from human ESCs (Kroon et al., 2008). In principle, the in vivo niche can provide all essential factors that support the functional maintenance of target cell types, thus facilitating functional lineage conversion. For instance, although the induction of murine cardiomyocyte-like cells by the overexpression of *Gata4*, *Mef2c*, and *Tbx5* was inefficient in vitro (Ieda et al., 2010), the overexpression of the same three factors in murine cardiac fibroblasts in vivo resulted in the robust generation of functional cardiomyocyte-like cells (Qian et al., 2012); this finding suggests the importance of the in vivo niche during the conversion process.

Induction of Lineage Subtypes with Specific Function. For target lineages composed of cell subtypes, the induction of a generic lineage-specific phenotype by exogenous factors is typically not sufficient for the generation of fully mature converted cells with diversified functions. In this regard, the induction of the neuronal lineage, which is constituted by a vast array of regional specific neuronal subtypes, may offer an instructive example. Although the originally identified neural factor combination BAM can convert fibroblasts into iN cells with basic neuronal functional features (Vierbuchen et al., 2010), the converted cells often exhibit glutamatergic neuronal properties, which have limited applicative potentials in the studies of neural diseases caused by certain neuronal subpopulations, such as Parkinson's disease. Consequently, different functional neuronal subtypes have been generated using lineage reprogramming, including dopaminergic (DA) neurons and motor neurons (MNs) (Caiazzo et al., 2011; Kim et al., 2011b; Liu et al., 2012; Pfisterer et al., 2011; Sheng et al., 2012a; Son et al., 2011). These iN subtypes not only possess fundamental neuronal functional properties but also exhibit subtype-specific functional features that resemble their in vivo counterparts such as the secretion of dopamine in DA neurons (Caiazzo et al., 2011; Kim et al., 2011b; Liu et al., 2012).

Notably, the addition of subtype-specific factors to factors that induce the generic neuronal conversion has been shown to induce neuronal subtypes. For instance, the combination of BAM factors and subtype-specific transcription factors that regulate motor neuron development enabled the induction of functional motor neurons from mouse and human fibroblasts (Son et al., 2011). This approach may facilitate the generation of subtypes in other lineages, thereby combining the factors that are responsible for generic lineage induction and subtype-specific factors. Considering that the functional heterogeneity of cell subtypes is a common phenomenon for multiple lineages, such as cardiomyocytes and hepatocytes (Evans et al., 2010; Gebhardt, 1992), the induction of functional lineage subtypes could have many applications for solving the functional maturation problem for a broad panel of lineages other than neuronal cells.

Advances in the Optimization of Assays for Induced Cell Characterization

Comparison with Primary Isolated Cells. One of the most critical issues in lineage reprogramming is to evaluate the similarity between the converted cells and the target cell type. Currently,

only several studies have included the primary isolated samples of target cell type as the positive control (Du et al., 2014; Huang et al., 2014; Riddell et al., 2014; Zhou et al., 2008), which makes it relatively difficult to compare the function of converted cells generated from different studies. Furthermore, the in vitro culture conditions for most somatic cell types are sub-optimized; thus, the cultivation of these positive controls may lead to the loss of their original features, which makes them inappropriate as a reference for converted cells. For instance, hepatic features can be quickly lost upon prolonged cultivation or undergoing the freeze-thaw cycle. Instead, freshly isolated primary hepatocytes maintain most features of the in vivo hepatocytes, and mixing these cells from different individuals can further reduce the potential variation caused by their heterogeneity of the genetic background. Accordingly, mixtures of freshly isolated primary hepatocytes from different individuals were used as the positive controls for hiHeps (Du et al., 2014). This example may show the importance of selecting proper positive controls for the evaluation of induced cell types by lineage reprogramming. In addition, proper positive controls can also facilitate the optimization of protocols for lineage induction, which serves as a reference for different reprogramming conditions.

Development of Novel Assays for the Analysis of Converted Cell Features. Different methods have been applied to the evaluation of converted cells (Cohen and Melton, 2011), such as the analysis of marker gene expression, global gene expression and epigenetic patterns, the silencing of exogenous factors, and the epigenetic state of master genes. However, these assays are primarily based on the whole cell population, which cannot reveal the heterogeneity of converted cells. In this regard, single-cell analysis provides an important tool to address this issue, which has been used in the characterization of iN cells and iHSCs (Marro et al., 2011; Riddell et al., 2014). Currently, single-cell analysis is mainly used to analyze the expression of key marker genes in individual cells. However, further deep analysis that includes a highly multiplexed quantitative analysis and single-cell RNA sequencing could provide additional information on gene expression variation in the converted cells. These techniques have been applied to the analysis of mouse hematopoietic cells and human preimplantation embryos (Guo et al., 2013; Yan et al., 2013).

Another important issue is how to quantitatively define the extent of the similarities between the converted cells and their in vivo counterparts; this question is relatively difficult to address using current methodology for the characterization of induced lineages. More recently, a major advance toward this goal has been made via the construction of a novel network biology platform referred to as CellNet (Cahan et al., 2014). Using 3,419 publicly available gene expression profiles of diverse cell types and tissues, the groups of James Collins and George Daley reconstructed the GRN for 20 cell types and tissues, which can be scored. As a result, they could not only quantitatively compare the directly converted cells with their in vivo counterparts, but also improve the quality of converted cells by spotting the aberrant GRN (Morris et al., 2014). Figuratively, the application of GRN analysis in lineage reprogramming has provided a "global positioning system" for the induction of functional cells in regenerative medicine, which not only tells us how far the "distance" is between the induced cells and their in vivo counterparts but also shows how to drive the partially induced cells toward their in vivo counterpart. Remarkably, this strategy offers a blueprint for engineering cell identity that could be widely applied to both lineage reprogramming and directed differentiation. However, several limitations of this platform remain, such as the inability to distinguish cell subtypes and cellular heterogeneity. The combination of single-cell expression profiling and GRN analysis may provide a solution for these current limitations in future studies.

Evaluation of Cell Function In Vivo. For the analysis of converted cell function, the most stringent test is to evaluate their function in vivo. In general, the assessment of the ability of converted cells to survive and integrate into the in vivo environment is typically the first step. Although this assay has been done on several murine lineages induced by lineage reprogramming (Bredenkamp et al., 2014; Han et al., 2012; Huang et al., 2011; Kim et al., 2011b; Hiramatsu et al., 2011; Najm et al., 2013; Riddell et al., 2014; Ring et al., 2012; Sekiya and Suzuki, 2011; Son et al., 2011; Thier et al., 2012; Yang et al., 2013), only a few induced human cell types have been transplanted in vivo and have shown the ability of surviving and integrating into the in vivo environment such as hepatocytes (Du et al., 2014; Huang et al., 2014; Zhu et al., 2014b), multipotent hematopoietic progenitors (Sandler et al., 2014), neural crest cells (Kim et al., 2014), limbal stem cells (LSCs) (Ouyang et al., 2014), and endothelial cells (Ginsberg et al., 2012).

The in vivo function of converted cells can be further tested using animal disease models. One representative example is the functional evaluation of induced thymic epithelial cells (TECs) from mouse fibroblasts via the enforced expression of *Foxn1* (Bredenkamp et al., 2014). The transplantation of induced TECs led to the formation of a complete, fully organized, and functional thymus in vivo, which supported T cell development in the athymic *nude* mice (Bredenkamp et al., 2014). Although these functional assays have been used as an important reference to assess the functional maturation of converted cells, the complete elimination of target cells is often unachievable, and the in vivo environment is more complicated than in vitro conditions. Thus, positive outcomes that may be the result of multiple factors such as re-proliferation of residual target cells or the instructive effects that result from other cell types, both of which are not directly related to the function of the converted cells, should be interpreted cautiously. Furthermore, additional attention must be given to the animal model itself in the evaluation of human converted cells. The xeno-rejection response and the interspecific difference between animals and humans may play important roles during the transplantation of converted human cells.

Strategies to Produce Large Numbers of Induced Cells for Translational Purposes

Complementary to the iPSC technology, functional cells generated by lineage reprogramming are, in principle, expected to be widely used for biomedical applications, including disease modeling, drug development, and cell therapy (Vierbuchen and Wernig, 2011). However, one major limitation of conventional direct lineage conversion is that converted terminal functional cells typically have poor proliferative ability, which is a major barrier for applicative purposes that require large cell numbers. Recently, several novel strategies have been developed to resolve this problem (Figure 4). One emerging solution is to generate proliferative stem cells or progenitors/precursors by

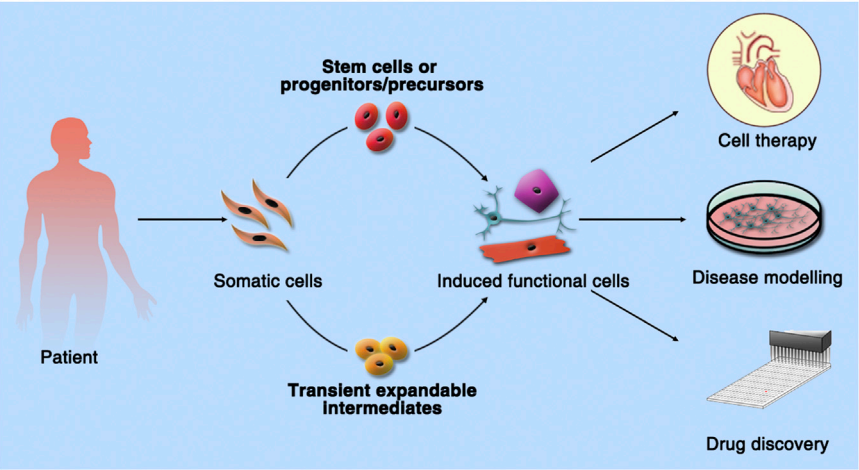

Figure 4. Novel Strategies for Large-Scale Induction of Lineage Conversion for Application Purposes

To generate a large number of converted cells for application purposes, somatic cells can be induced into stem cells or progenitor/precursors and propagated prior to further differentiation into terminally differentiated cells. Another strategy is to induce a transient proliferating intermediate state during lineage conversion, and these intermediates can gradually stop proliferation and become functional terminal cells.

lineage reprogramming. To some extent, stem cells or progenitors/precursors are more desirable for transplantation because they can efficiently engraft and integrate into the in vivo microenvironment. Recently, a number of stem cells or progenitors/precursors have been induced by lineage reprogramming such as neural stem cells or progenitors (Han et al., 2012; Kim et al., 2011a; Lujan et al., 2012; Ring et al., 2012; Sheng et al., 2012b; Thier et al., 2012), oligodendrocyte precursor cells (Najm et al., 2013; Yang et al., 2013), hepatic stem cells (Yu et al., 2013), HSCs (Riddell et al., 2014), and hematopoietic multipotent progenitors (Sandler et al., 2014). Notably, several types of stem cells or progenitors/precursors, such as neural stem cells or progenitors, have been shown to proliferate in vitro (Han et al., 2012; Kim et al., 2011a; Lujan et al., 2012; Ring et al., 2012; Sheng et al., 2012b; Thier et al., 2012), which suggests the potential of these cells for large-scale use.

In addition to the direct induction of stem cells or progenitors/precursors, another emerging potential strategy for obtaining large numbers of converted cells is to create a transient proliferative intermediate state during lineage conversion, which has been shown in the induction of human hepatocytes (Du et al., 2014). To overcome proliferation arrest and cell death during hepatic conversion, we employed *C-MYC* overexpression and *P53* knockdown in combination with the overexpression of hepatic transcription factors during the induction process. Strikingly, the inclusion of exogenous *C-MYC* and *P53* small interfering RNAs permitted the induction of proliferative intermediates with high expansion ability, which enabled a greater than 10^6-fold increase in cell number (Du et al., 2014). These intermediates can further become functionally mature upon the silencing of exogenous factors, which can lead to the production of large numbers of hepatocytes sufficient for applicative purposes such as drug discovery. It would be interesting to determine whether these factors can also be applied to the induction of transient proliferative intermediates during the induction of other cell types in the future.

Translation of Lineage Reprogramming toward Cell Therapy

Undoubtedly, the most exciting potential application of lineage reprogramming is cell replacement therapy (Figure 5). Several recent studies have, in principle, demonstrated the potential use of this strategy for generating cells for therapeutic purposes. To develop a novel approach for the treatment of corneal surface diseases, Ouyang et al. (2014) induced human LSCs from skin epithelial stem cells via the overexpression of *PAX6* , which can differentiate into cornea epithelial cells (CECs) in vivo. Impressively, when transplanted onto eyes in a rabbit corneal injury model, these reprogrammed LSCs could replenish CECs and repair the damaged corneal surface (Ouyang et al., 2014). Therefore, combined with novel strategies that expand converted cells as previously discussed, the induction of cells with therapeutic potential by direct conversion could be applied for clinical transplantation in the future.

Compared with the directed differentiation from pluripotent stem cells, lineage reprogramming has one obvious advantageous feature in that it can be conducted in vivo. The in vivo execution of this strategy could theoretically avoid the risk of teratoma formation and genetic mutations caused by long term in vitro culture. Furthermore, in vivo lineage reprogramming could also bypass the difficulties of transplantation, which is a potential problem in the application of cells induced in vitro by directed differentiation or lineage reprogramming. Finally, natural lineage conversions have been observed in vivo, such as the transdifferentiation of pancreatic α or δ cells into β cells after near-total β cell loss (Chera et al., 2014; Thorel et al., 2010). As a result, in vivo lineage reprogramming may represent a direct and simple approach to generating functional cells for replacement therapy.

Inspiringly, an increasing number of reports have demonstrated that lineage reprogramming can be achieved in vivo (Guo et al., 2014; Inagawa et al., 2012; Jayawardena et al., 2012; Li et al., 2014b; Mathison et al., 2012; Montana et al., 2013; Niu et al., 2013; Qian et al., 2012; Rouaux and Arlotta, 2013; Song et al., 2012; Torper et al., 2013; Zhou et al., 2008). Recently, it has been reported that glial cells, especially astrocytes, can be converted into functional neuroblasts or neurons in vivo (Guo et al., 2014; Niu et al., 2013; Torper et al., 2013). For instance, in Alzheimer's disease mouse models, Guo et al. (2014) successfully reprogrammed reactive glial cells into functional neurons, suggesting a potential application of in vivo reprogramming for brain repair. The recent induction of cardiomyocytes from cardiac fibroblasts in vivo is another step in the efforts that aim to develop novel therapies using in vivo lineage reprogramming (Qian et al., 2012; Song et al., 2012). Through the delivery of the cardiac reprogramming factors into the mouse

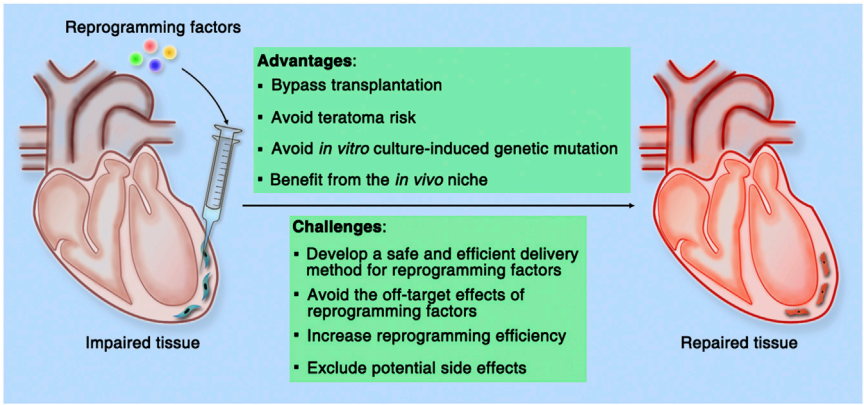

Figure 5. In Vivo Lineage Reprogramming for Cell Therapy

Reprogramming factors can be delivered into the target in vivo site to induce the conversion of somatic cells into the desired cells, thereby repairing the impaired tissue in situ. The advantages and challenges of this strategy are highlighted.

heart after coronary ligation, Qian et al. (2012) demonstrated that approximately 35% of the cardiomyocytes in the border/infarct zone were induced from cardiac fibroblasts. Importantly, cardiac function was significantly improved in reprogramming-factor-infected mice compared with controls. In another study performed by Song et al. (2012), cardiomyocyte-like cells were induced from endogenous cardiac fibroblasts by lineage reprogramming in the injured heart. Cardiac function was enhanced by the injection of reprogramming factors compared with control mice. Although these studies have, in principle, demonstrated that in vivo lineage reprogramming has therapeutic effects in disease models, the exact role of the in situ converted cells during this process remains unknown and requires further exploration of the mechanism for the observed therapeutic effects.

Despite great promise for the treatment of diseases, the in vivo application of lineage reprogramming must still overcome several major hurdles. For instance, a safe and efficient delivery method must be established for the delivery of the reprogramming factors to the targeted in vivo sites. Avoidance of the off-target effects of reprogramming factors in vivo is also an important issue, because these effects may induce unwanted cells. Finally, the potential side effects of in vivo reprogramming must be studied in detail and further excluded. For example, the potential risk of arrhythmias may arise if the cardiomyocytes generated by the in vivo reprogramming approach exhibit electrical heterogeneity (Xu et al., 2012).

Perspectives

With rapid progress that includes not only the identification of new factors but also the development of novel strategies for the generation of functionally mature cells, the initial stage of studies in lineage reprogramming that focused on demonstrating the feasibility of this strategy has passed. Now, we are progressing toward the next generation of lineage reprogramming for which a systematic guidebook for engineering different cellular states is progressively being built. Although it was originally thought to be difficult to scale up the cells generated by lineage reprogramming (Vierbuchen and Wernig, 2011), emerging approaches, such as the conversion into proliferative stem cells or progenitors/precursors and the induction of expandable transient intermediates, permit the potential use of lineage reprogramming for biomedical applications that require a large number of cells. These significant advances have undoubtedly made lineage reprogramming a complementary strategy for directed differentiation from pluripotent stem cells for providing cell resources for regenerative medicine.

Although the field of lineage reprogramming has progressed rapidly in recent years, it is obvious that it still faces several key challenges for its use in both research and therapeutic applications. First, the functional maturation of converted cells remains a fundamental issue for the entire field. In general, the acquisition of functional features is a slow and inefficient process that results in functional heterogeneity of the converted cells. Thus, significant work is needed to optimize current protocols for the production of fully functional cells with a highly purity. Second, more efficient and robust strategies for converting mature cell types require a better understanding of the mechanism of lineage reprogramming. Answers to these questions will facilitate the generation of functionally mature cells and will benefit by developing our understanding of the principles of cell fate determination. Finally, for therapeutic purposes, substantially safer approaches are required that will avoid genetic manipulation and the use of animal-derived factors. In this regard, the use of small molecules for developing chemically defined protocols would be one promising way for the clinical application of lineage reprogramming. It is also essential to develop detailed global analysis to guarantee the integration and fidelity of converted cells to their in vivo counterparts on genetic and epigenetic levels. Once the challenges previously discussed are overcome, we anticipate that lineage reprogramming will ultimately enable the generation of any desired cell state both in vitro and in vivo as well as the recapitulation of complex tissues that consist of multiple lineages in the future.

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REFERENCES

Addis, R.C., Ifkovits, J.L., Pinto, F., Kellam, L.D., Esteso, P., Rentschler, S., Christoforou, N., Epstein, J.A., and Gearhart, J.D. (2013). Optimization of direct fibroblast reprogramming to cardiomyocytes using calcium activity as a functional measure of success. J. Mol. Cell. Cardiol. *60*, 97–106.

Ambasudhan, R., Talantova, M., Coleman, R., Yuan, X., Zhu, S., Lipton, S.A., and Ding, S. (2011). Direct reprogramming of adult human fibroblasts to functional neurons under defined conditions. Cell Stem Cell *9*, 113–118.

Bartel, D.P. (2009). MicroRNAs: target recognition and regulatory functions. Cell *136*, 215–233.

Batta, K., Florkowska, M., Kouskoff, V., and Lacaud, G. (2014). Direct reprogramming of murine fibroblasts to hematopoietic progenitor cells. Cell Rep. *9*, 1871–1884.

Bhatia, S.N., Underhill, G.H., Zaret, K.S., and Fox, I.J. (2014). Cell and tissue engineering for liver disease. Sci. Transl. Med. *6*, http://dx.doi.org/10.1126/scitranslmed.3005975.

Bredenkamp, N., Ulyanchenko, S., O'Neill, K.E., Manley, N.R., Vaidya, H.J., and Blackburn, C.C. (2014). An organized and functional thymus generated from FOXN1-reprogrammed fibroblasts. Nat. Cell Biol. *16*, 902–908.

Buganim, Y., Itskovich, E., Hu, Y.C., Cheng, A.W., Ganz, K., Sarkar, S., Fu, D., Welstead, G.G., Page, D.C., and Jaenisch, R. (2012). Direct reprogramming of fibroblasts into embryonic Sertoli-like cells by defined factors. Cell Stem Cell *11*, 373–386.

Buganim, Y., Faddah, D.A., and Jaenisch, R. (2013). Mechanisms and models of somatic cell reprogramming. Nat. Rev. Genet. *14*, 427–439.

Bussmann, L.H., Schubert, A., Vu Manh, T.P., De Andres, L., Desbordes, S.C., Parra, M., Zimmermann, T., Rapino, F., Rodriguez-Ubreva, J., Ballestar, E., and Graf, T. (2009). A robust and highly efficient immune cell reprogramming system. Cell Stem Cell *5*, 554–566.

Cahan, P., Li, H., Morris, S.A., Lummertz da Rocha, E., Daley, G.Q., and Collins, J.J. (2014). CellNet: network biology applied to stem cell engineering. Cell *158*, 903–915.

Caiazzo, M., Dell'Anno, M.T., Dvoretskova, E., Lazarevic, D., Taverna, S., Leo, D., Sotnikova, T.D., Menegon, A., Roncaglia, P., Colciago, G., et al. (2011). Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. Nature *476*, 224–227.

Caiazzo, M., Giannelli, S., Valente, P., Lignani, G., Carissimo, A., Sessa, A., Colasante, G., Bartolomeo, R., Massimino, L., Ferroni, S., et al. (2014). Direct Conversion of Fibroblasts into Functional Astrocytes by Defined Transcription Factors. Stem Cell Reports *4*, 25–36.

Carey, B.W., Markoulaki, S., Hanna, J.H., Faddah, D.A., Buganim, Y., Kim, J., Ganz, K., Steine, E.J., Cassady, J.P., Creyghton, M.P., et al. (2011). Reprogramming factor stoichiometry influences the epigenetic state and biological properties of induced pluripotent stem cells. Cell Stem Cell *9*, 588–598.

Chanda, S., Ang, C.E., Davila, J., Pak, C., Mall, M., Lee, Q.Y., Ahlenius, H., Jung, S.W., Südhof, T.C., and Wernig, M. (2014). Generation of induced neuronal cells by the single reprogramming factor ASCL1. Stem Cell Reports *3*, 282–296.

Cheng, L., Hu, W., Qiu, B., Zhao, J., Yu, Y., Guan, W., Wang, M., Yang, W., and Pei, G. (2014). Generation of neural progenitor cells by chemical cocktails and hypoxia. Cell Res. *24*, 665–679.

Chera, S., Baronnier, D., Ghila, L., Cigliola, V., Jensen, J.N., Gu, G., Furuyama, K., Thorel, F., Gribble, F.M., Reimann, F., and Herrera, P.L. (2014). Diabetes recovery by age-dependent conversion of pancreatic δ-cells into insulin producers. Nature *514*, 503–507.

Christoforou, N., Chellappan, M., Adler, A.F., Kirkton, R.D., Wu, T., Addis, R.C., Bursac, N., and Leong, K.W. (2013). Transcription factors MYOCD, SRF, Mesp1 and SMARCD3 enhance the cardio-inducing effect of GATA4, TBX5, and MEF2C during direct cellular reprogramming. PLoS ONE *8*, e63577.

Cohen, D.E., and Melton, D. (2011). Turning straw into gold: directing cell fate for regenerative medicine. Nat. Rev. Genet. *12*, 243–252.

Corti, S., Nizzardo, M., Simone, C., Falcone, M., Donadoni, C., Salani, S., Rizzo, F., Nardini, M., Riboldi, G., Magri, F., et al. (2012). Direct reprogramming of human astrocytes into neural stem cells and neurons. Exp. Cell Res. *318*, 1528–1541.

Courtney, M., Gjernes, E., Druelle, N., Ravaud, C., Vieira, A., Ben-Othman, N., Pfeifer, A., Avolio, F., Leuckx, G., Lacas-Gervais, S., et al. (2013). The inactivation of Arx in pancreatic α-cells triggers their neogenesis and conversion into functional β-like cells. PLoS Genet. *9*, e1003934.

Davis, R.L., Weintraub, H., and Lassar, A.B. (1987). Expression of a single transfected cDNA converts fibroblasts to myoblasts. Cell *51*, 987–1000.

Dhawan, S., Georgia, S., Tschen, S.I., Fan, G., and Bhushan, A. (2011). Pancreatic β cell identity is maintained by DNA methylation-mediated repression of Arx. Dev. Cell *20*, 419–429.

Du, Y., Wang, J., Jia, J., Song, N., Xiang, C., Xu, J., Hou, Z., Su, X., Liu, B., Jiang, T., et al. (2014). Human hepatocytes with drug metabolic function induced from fibroblasts by lineage reprogramming. Cell Stem Cell *14*, 394–403.

Dunn, S.J., Martello, G., Yordanov, B., Emmott, S., and Smith, A.G. (2014). Defining an essential transcription factor program for naïve pluripotency. Science *344*, 1156–1160.

Efe, J.A., Hilcove, S., Kim, J., Zhou, H., Ouyang, K., Wang, G., Chen, J., and Ding, S. (2011). Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy. Nat. Cell Biol. *13*, 215–222.

Evans, S.M., Yelon, D., Conlon, F.L., and Kirby, M.L. (2010). Myocardial lineage development. Circ. Res. *107*, 1428–1444.

Feng, R., Desbordes, S.C., Xie, H., Tillo, E.S., Pixley, F., Stanley, E.R., and Graf, T. (2008). PU.1 and C/EBPalpha/beta convert fibroblasts into macrophage-like cells. Proc. Natl. Acad. Sci. USA *105*, 6057–6062.

Forsberg, M., Carlén, M., Meletis, K., Yeung, M.S., Barnabé-Heider, F., Persson, M.A., Aarum, J., and Frisén, J. (2010). Efficient reprogramming of adult neural stem cells to monocytes by ectopic expression of a single gene. Proc. Natl. Acad. Sci. USA *107*, 14657–14661.

Fu, J.D., Stone, N.R., Liu, L., Spencer, C.I., Qian, L., Hayashi, Y., Delgado-Olguin, P., Ding, S., Bruneau, B.G., and Srivastava, D. (2013). Direct reprogramming of human fibroblasts toward a cardiomyocyte-like state. Stem Cell Reports *1*, 235–247.

Gebhardt, R. (1992). Metabolic zonation of the liver: regulation and implications for liver function. Pharmacol. Ther. *53*, 275–354.

Ginsberg, M., James, D., Ding, B.S., Nolan, D., Geng, F., Butler, J.M., Schachterle, W., Pulijaal, V.R., Mathew, S., Chasen, S.T., et al. (2012). Efficient direct reprogramming of mature amniotic cells into endothelial cells by ETS factors and TGFβ suppression. Cell *151*, 559–575.

Graf, T., and Enver, T. (2009). Forcing cells to change lineages. Nature *462*, 587–594.

Guo, G., Luc, S., Marco, E., Lin, T.W., Peng, C., Kerenyi, M.A., Beyaz, S., Kim, W., Xu, J., Das, P.P., et al. (2013). Mapping cellular hierarchy by single-cell analysis of the cell surface repertoire. Cell Stem Cell *13*, 492–505.

Guo, Z., Zhang, L., Wu, Z., Chen, Y., Wang, F., and Chen, G. (2014). In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model. Cell Stem Cell *14*, 188–202.

Han, D.W., Tapia, N., Hermann, A., Hemmer, K., Höing, S., Araúzo-Bravo, M.J., Zaehres, H., Wu, G., Frank, S., Moritz, S., et al. (2012). Direct reprogramming of fibroblasts into neural stem cells by defined factors. Cell Stem Cell *10*, 465–472.

Han, J.K., Chang, S.H., Cho, H.J., Choi, S.B., Ahn, H.S., Lee, J., Jeong, H., Youn, S.W., Lee, H.J., Kwon, Y.W., et al. (2014). Direct conversion of adult skin fibroblasts to endothelial cells by defined factors. Circulation *130*, 1168–1178.

Hayashi, T., Mizuno, N., Takada, R., Takada, S., and Kondoh, H. (2006). Determinative role of Wnt signals in dorsal iris-derived lens regeneration in newt eye. Mech. Dev. *123*, 793–800.

Heinrich, C., Blum, R., Gascón, S., Masserdotti, G., Tripathi, P., Sánchez, R., Tiedt, S., Schroeder, T., Götz, M., and Berninger, B. (2010). Directing astroglia from the cerebral cortex into subtype specific functional neurons. PLoS Biol. *8*, e1000373.

Heins, N., Malatesta, P., Cecconi, F., Nakafuku, M., Tucker, K.L., Hack, M.A., Chapouton, P., Barde, Y.A., and Götz, M. (2002). Glial cells generate neurons: the role of the transcription factor Pax6. Nat. Neurosci. *5*, 308–315.

Hendry, C.E., Vanslambrouck, J.M., Ineson, J., Suhaimi, N., Takasato, M., Rae, F., and Little, M.H. (2013). Direct transcriptional reprogramming of adult cells to embryonic nephron progenitors. J. Am. Soc. Nephrol. *24*, 1424–1434.

Heyworth, C., Pearson, S., May, G., and Enver, T. (2002). Transcription factor-mediated lineage switching reveals plasticity in primary committed progenitor cells. EMBO J. *21*, 3770–3781.

Hiramatsu, K., Sasagawa, S., Outani, H., Nakagawa, K., Yoshikawa, H., and Tsumaki, N. (2011). Generation of hyaline cartilaginous tissue from mouse adult dermal fibroblast culture by defined factors. J. Clin. Invest. *121*, 640–657.

Hou, P.P., Li, Y.Q., Zhang, X., Liu, C., Guan, J.Y., Li, H.G., Zhao, T., Ye, J.Q., Yang, W.F., Liu, K., et al. (2013). Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. Science *341*, 651–654.

Huang, X., and Wang, J. (2014). The extended pluripotency protein interactome and its links to reprogramming. Curr. Opin. Genet. Dev. *28C*, 16–24.

Huang, P., He, Z., Ji, S., Sun, H., Xiang, D., Liu, C., Hu, Y., Wang, X., and Hui, L. (2011). Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. Nature *475*, 386–389.

Huang, P., Zhang, L., Gao, Y., He, Z., Yao, D., Wu, Z., Cen, J., Chen, X., Liu, C., Hu, Y., et al. (2014). Direct reprogramming of human fibroblasts to functional and expandable hepatocytes. Cell Stem Cell *14*, 370–384.

Ieda, M., Fu, J.D., Delgado-Olguin, P., Vedantham, V., Hayashi, Y., Bruneau, B.G., and Srivastava, D. (2010). Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell *142*, 375–386.

Inagawa, K., Miyamoto, K., Yamakawa, H., Muraoka, N., Sadahiro, T., Umei, T., Wada, R., Katsumata, Y., Kaneda, R., Nakade, K., et al. (2012). Induction of cardiomyocyte-like cells in infarct hearts by gene transfer of Gata4, Mef2c, and Tbx5. Circ. Res. *111*, 1147–1156.

Ionta, V., Liang, W., Kim, E.H., Rafie, R., Giacomello, A., Marbán, E., and Cho, H.C. (2015). SHOX2 Overexpression Favors Differentiation of Embryonic Stem Cells into Cardiac Pacemaker Cells, Improving Biological Pacing Ability. Stem Cell Reports *4*, 129–142.

Islas, J.F., Liu, Y., Weng, K.C., Robertson, M.J., Zhang, S., Prejusa, A., Harger, J., Tikhomirova, D., Chopra, M., Iyer, D., et al. (2012). Transcription factors ETS2 and MESP1 transdifferentiate human dermal fibroblasts into cardiac progenitors. Proc. Natl. Acad. Sci. USA *109*, 13016–13021.

Jayawardena, T.M., Egemnazarov, B., Finch, E.A., Zhang, L., Payne, J.A., Pandya, K., Zhang, Z., Rosenberg, P., Mirotsou, M., and Dzau, V.J. (2012). MicroRNA-mediated in vitro and in vivo direct reprogramming of cardiac fibroblasts to cardiomyocytes. Circ. Res. *110*, 1465–1473.

Jenuwein, T., and Allis, C.D. (2001). Translating the histone code. Science *293*, 1074–1080.

Jopling, C., Boue, S., and Izpisua Belmonte, J.C. (2011). Dedifferentiation, transdifferentiation and reprogramming: three routes to regeneration. Nat. Rev. Mol. Cell Biol. *12*, 79–89.

Kajimura, S., Seale, P., Kubota, K., Lunsford, E., Frangioni, J.V., Gygi, S.P., and Spiegelman, B.M. (2009). Initiation of myoblast to brown fat switch by a PRDM16-C/EBP-beta transcriptional complex. Nature *460*, 1154–1158.

Kaneto, H., Nakatani, Y., Miyatsuka, T., Matsuoka, T.A., Matsuhisa, M., Hori, M., and Yamasaki, Y. (2005). PDX-1/VP16 fusion protein, together with NeuroD or Ngn3, markedly induces insulin gene transcription and ameliorates glucose tolerance. Diabetes *54*, 1009–1022.

Karow, M., Sánchez, R., Schichor, C., Masserdotti, G., Ortega, F., Heinrich, C., Gascón, S., Khan, M.A., Lie, D.C., Dellavalle, A., et al. (2012). Reprogramming of pericyte-derived cells of the adult human brain into induced neuronal cells. Cell Stem Cell *11*, 471–476.

Kim, J., Efe, J.A., Zhu, S., Talantova, M., Yuan, X., Wang, S., Lipton, S.A., Zhang, K., and Ding, S. (2011a). Direct reprogramming of mouse fibroblasts to neural progenitors. Proc. Natl. Acad. Sci. USA *108*, 7838–7843.

Kim, J., Su, S.C., Wang, H., Cheng, A.W., Cassady, J.P., Lodato, M.A., Lengner, C.J., Chung, C.Y., Dawlaty, M.M., Tsai, L.H., and Jaenisch, R. (2011b). Functional integration of dopaminergic neurons directly converted from mouse fibroblasts. Cell Stem Cell *9*, 413–419.

Kim, Y.J., Lim, H., Li, Z., Oh, Y., Kovlyagina, I., Choi, I.Y., Dong, X., and Lee, G. (2014). Generation of multipotent induced neural crest by direct reprogramming of human postnatal fibroblasts with a single transcription factor. Cell Stem Cell *15*, 497–506.

Kroon, E., Martinson, L.A., Kadoya, K., Bang, A.G., Kelly, O.G., Eliazer, S., Young, H., Richardson, M., Smart, N.G., Cunningham, J., et al. (2008). Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat. Biotechnol. *26*, 443–452.

Kulessa, H., Frampton, J., and Graf, T. (1995). GATA-1 reprograms avian myelomonocytic cell lines into eosinophils, thromboblasts, and erythroblasts. Genes Dev. *9*, 1250–1262.

Kurian, L., Sancho-Martinez, I., Nivet, E., Aguirre, A., Moon, K., Pendaries, C., Volle-Challier, C., Bono, F., Herbert, J.M., Pulecio, J., et al. (2013). Conversion of human fibroblasts to angioblast-like progenitor cells. Nat. Methods *10*, 77–83.

Kyba, M., Perlingeiro, R.C., and Daley, G.Q. (2002). HoxB4 confers definitive lymphoid-myeloid engraftment potential on embryonic stem cell and yolk sac hematopoietic progenitors. Cell *109*, 29–37.

Ladewig, J., Mertens, J., Kesavan, J., Doerr, J., Poppe, D., Glaue, F., Herms, S., Wernet, P., Kögler, G., Müller, F.J., et al. (2012). Small molecules enable highly efficient neuronal conversion of human fibroblasts. Nat. Methods *9*, 575–578.

Lemper, M., Leuckx, G., Heremans, Y., German, M.S., Heimberg, H., Bouwens, L., and Baeyens, L. (2014). Reprogramming of human pancreatic exocrine cells to β-like cells. Cell Death Differ.. http://dx.doi.org/10.1038/cdd.2014.193.

Li, P., Burke, S., Wang, J., Chen, X., Ortiz, M., Lee, S.C., Lu, D., Campos, L., Goulding, D., Ng, B.L., et al. (2010). Reprogramming of T cells to natural killer-like cells upon Bcl11b deletion. Science *329*, 85–89.

Li, J., Huang, N.F., Zou, J., Laurent, T.J., Lee, J.C., Okogbaa, J., Cooke, J.P., and Ding, S. (2013a). Conversion of human fibroblasts to functional endothelial cells by defined factors. Arterioscler. Thromb. Vasc. Biol. *33*, 1366–1375.

Li, W.L., Li, K., Wei, W.G., and Ding, S. (2013b). Chemical approaches to stem cell biology and therapeutics. Cell Stem Cell *13*, 270–283.

Li, K., Zhu, S., Russ, H.A., Xu, S., Xu, T., Zhang, Y., Ma, T., Hebrok, M., and Ding, S. (2014a). Small molecules facilitate the reprogramming of mouse fibroblasts into pancreatic lineages. Cell Stem Cell *14*, 228–236.

Li, W., Nakanishi, M., Zumsteg, A., Shear, M., Wright, C., Melton, D.A., and Zhou, Q. (2014b). In vivo reprogramming of pancreatic acinar cells to three islet endocrine subtypes. Elife *3*, e01846.

Lim, L.P., Lau, N.C., Garrett-Engele, P., Grimson, A., Schelter, J.M., Castle, J., Bartel, D.P., Linsley, P.S., and Johnson, J.M. (2005). Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature *433*, 769–773.

Liu, X., Li, F., Stubblefield, E.A., Blanchard, B., Richards, T.L., Larson, G.A., He, Y., Huang, Q., Tan, A.C., Zhang, D., et al. (2012). Direct reprogramming of human fibroblasts into dopaminergic neuron-like cells. Cell Res. *22*, 321–332.

Liu, M.L., Zang, T., Zou, Y., Chang, J.C., Gibson, J.R., Huber, K.M., and Zhang, C.L. (2013). Small molecules enable neurogenin 2 to efficiently convert human fibroblasts into cholinergic neurons. Nat. Commun. *4*, 2183.

Loh, K.M., and Lim, B. (2011). A precarious balance: pluripotency factors as lineage specifiers. Cell Stem Cell *8*, 363–369.

Lujan, E., Chanda, S., Ahlenius, H., Südhof, T.C., and Wernig, M. (2012). Direct conversion of mouse fibroblasts to self-renewing, tripotent neural precursor cells. Proc. Natl. Acad. Sci. USA *109*, 2527–2532.

Ma, T., Xie, M., Laurent, T., and Ding, S. (2013). Progress in the reprogramming of somatic cells. Circ. Res. *112*, 562–574.

Maki, N., Martinson, J., Nishimura, O., Tarui, H., Meller, J., Tsonis, P.A., and Agata, K. (2010). Expression profiles during dedifferentiation in newt lens regeneration revealed by expressed sequence tags. Mol. Vis. *16*, 72–78.

Marro, S., Pang, Z.P., Yang, N., Tsai, M.C., Qu, K., Chang, H.Y., Südhof, T.C., and Wernig, M. (2011). Direct lineage conversion of terminally differentiated hepatocytes to functional neurons. Cell Stem Cell *9*, 374–382.

Martinat, C., Bacci, J.J., Leete, T., Kim, J., Vanti, W.B., Newman, A.H., Cha, J.H., Gether, U., Wang, H.G., and Abeliovich, A. (2006). Cooperative transcription activation by Nurr1 and Pitx3 induces embryonic stem cell maturation to

the midbrain dopamine neuron phenotype. Proc. Natl. Acad. Sci. USA *103*, 2874–2879.

Mathison, M., Gersch, R.P., Nasser, A., Lilo, S., Korman, M., Fourman, M., Hackett, N., Shroyer, K., Yang, J., Ma, Y., et al. (2012). In vivo cardiac cellular reprogramming efficacy is enhanced by angiogenic preconditioning of the infarcted myocardium with vascular endothelial growth factor. J. Am. Heart Assoc. *1*, e005652.

Montana, C.L., Kolesnikov, A.V., Shen, S.Q., Myers, C.A., Kefalov, V.J., and Corbo, J.C. (2013). Reprogramming of adult rod photoreceptors prevents retinal degeneration. Proc. Natl. Acad. Sci. USA *110*, 1732–1737.

Morris, S.A., Cahan, P., Li, H., Zhao, A.M., San Roman, A.K., Shivdasani, R.A., Collins, J.J., and Daley, G.Q. (2014). Dissecting engineered cell types and enhancing cell fate conversion via CellNet. Cell *158*, 889–902.

Murry, C.E., and Keller, G. (2008). Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. Cell *132*, 661–680.

Najm, F.J., Lager, A.M., Zaremba, A., Wyatt, K., Caprariello, A.V., Factor, D.C., Karl, R.T., Maeda, T., Miller, R.H., and Tesar, P.J. (2013). Transcription factor-mediated reprogramming of fibroblasts to expandable, myelinogenic oligodendrocyte progenitor cells. Nat. Biotechnol. *31*, 426–433.

Nam, Y.J., Song, K., Luo, X., Daniel, E., Lambeth, K., West, K., Hill, J.A., DiMaio, J.M., Baker, L.A., Bassel-Duby, R., and Olson, E.N. (2013). Reprogramming of human fibroblasts toward a cardiac fate. Proc. Natl. Acad. Sci. USA *110*, 5588–5593.

Neo, W.H., Yap, K., Lee, S.H., Looi, L.S., Khandelia, P., Neo, S.X., Makeyev, E.V., and Su, I.H. (2014). MicroRNA miR-124 controls the choice between neuronal and astrocyte differentiation by fine-tuning Ezh2 expression. J. Biol. Chem. *289*, 20788–20801.

Nerlov, C., and Graf, T. (1998). PU.1 induces myeloid lineage commitment in multipotent hematopoietic progenitors. Genes Dev. *12*, 2403–2412.

Niu, W., Zang, T., Zou, Y., Fang, S., Smith, D.K., Bachoo, R., and Zhang, C.L. (2013). In vivo reprogramming of astrocytes to neuroblasts in the adult brain. Nat. Cell Biol. *15*, 1164–1175.

Niwa, H., Miyazaki, J., and Smith, A.G. (2000). Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat. Genet. *24*, 372–376.

Oberstrass, F.C., Auweter, S.D., Erat, M., Hargous, Y., Henning, A., Wenter, P., Reymond, L., Amir-Ahmady, B., Pitsch, S., Black, D.L., and Allain, F.H. (2005). Structure of PTB bound to RNA: specific binding and implications for splicing regulation. Science *309*, 2054–2057.

Ouyang, H., Xue, Y., Lin, Y., Zhang, X., Xi, L., Patel, S., Cai, H., Luo, J., Zhang, M., Zhang, M., et al. (2014). WNT7A and PAX6 define corneal epithelium homeostasis and pathogenesis. Nature *511*, 358–361.

Pang, Z.P., Yang, N., Vierbuchen, T., Ostermeier, A., Fuentes, D.R., Yang, T.Q., Citri, A., Sebastiano, V., Marro, S., Südhof, T.C., and Wernig, M. (2011). Induction of human neuronal cells by defined transcription factors. Nature *476*, 220–223.

Papapetrou, E.P., Tomishima, M.J., Chambers, S.M., Mica, Y., Reed, E., Menon, J., Tabar, V., Mo, Q.X., Studer, L., and Sadelain, M. (2009). Stoichiometric and temporal requirements of Oct4, Sox2, Klf4, and c-Myc expression for efficient human iPSC induction and differentiation. Proc. Natl. Acad. Sci. USA *106*, 12759–12764.

Patel, T., Tursun, B., Rahe, D.P., and Hobert, O. (2012). Removal of Polycomb repressive complex 2 makes C. elegans germ cells susceptible to direct conversion into specific somatic cell types. Cell Rep. *2*, 1178–1186.

Pereira, C.F., Chang, B., Qiu, J., Niu, X., Papatsenko, D., Hendry, C.E., Clark, N.R., Nomura-Kitabayashi, A., Kovacic, J.C., Ma'ayan, A., et al. (2013). Induction of a hemogenic program in mouse fibroblasts. Cell Stem Cell *13*, 205–218.

Pfisterer, U., Kirkeby, A., Torper, O., Wood, J., Nelander, J., Dufour, A., Björklund, A., Lindvall, O., Jakobsson, J., and Parmar, M. (2011). Direct conversion of human fibroblasts to dopaminergic neurons. Proc. Natl. Acad. Sci. USA *108*, 10343–10348.

Protze, S., Khattak, S., Poulet, C., Lindemann, D., Tanaka, E.M., and Ravens, U. (2012). A new approach to transcription factor screening for reprogramming of fibroblasts to cardiomyocyte-like cells. J. Mol. Cell. Cardiol. *53*, 323–332.

Pulecio, J., Nivet, E., Sancho-Martinez, I., Vitaloni, M., Guenechea, G., Xia, Y., Kurian, L., Dubova, I., Bueren, J., Laricchia-Robbio, L., and Izpisua Belmonte, J.C. (2014). Conversion of human fibroblasts into monocyte-like progenitor cells. Stem Cells *32*, 2923–2938.

Qian, L., Huang, Y., Spencer, C.I., Foley, A., Vedantham, V., Liu, L., Conway, S.J., Fu, J.D., and Srivastava, D. (2012). In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. Nature *485*, 593–598.

Riddell, J., Gazit, R., Garrison, B.S., Guo, G., Saadatpour, A., Mandal, P.K., Ebina, W., Volchkov, P., Yuan, G.C., Orkin, S.H., and Rossi, D.J. (2014). Reprogramming committed murine blood cells to induced hematopoietic stem cells with defined factors. Cell *157*, 549–564.

Ring, K.L., Tong, L.M., Balestra, M.E., Javier, R., Andrews-Zwilling, Y., Li, G., Walker, D., Zhang, W.R., Kreitzer, A.C., and Huang, Y. (2012). Direct reprogramming of mouse and human fibroblasts into multipotent neural stem cells with a single factor. Cell Stem Cell *11*, 100–109.

Rouaux, C., and Arlotta, P. (2013). Direct lineage reprogramming of post-mitotic callosal neurons into corticofugal neurons in vivo. Nat. Cell Biol. *15*, 214–221.

Sandler, V.M., Lis, R., Liu, Y., Kedem, A., James, D., Elemento, O., Butler, J.M., Scandura, J.M., and Rafii, S. (2014). Reprogramming human endothelial cells to haematopoietic cells requires vascular induction. Nature *511*, 312–318.

Sayed, N., Wong, W.T., Ospino, F., Meng, S., Lee, J., Jha, A., Dexheimer, P., Aronow, B.J., and Cooke, J.P. (2015). Transdifferentiation of human fibroblasts to endothelial cells: role of innate immunity. Circulation *131*, 300–309.

Sekiya, S., and Suzuki, A. (2011). Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors. Nature *475*, 390–393.

Shen, C.N., Slack, J.M., and Tosh, D. (2000). Molecular basis of transdifferentiation of pancreas to liver. Nat. Cell Biol. *2*, 879–887.

Sheng, C., Zheng, Q., Wu, J., Xu, Z., Sang, L., Wang, L., Guo, C., Zhu, W., Tong, M., Liu, L., et al. (2012a). Generation of dopaminergic neurons directly from mouse fibroblasts and fibroblast-derived neural progenitors. Cell Res. *22*, 769–772.

Sheng, C., Zheng, Q., Wu, J., Xu, Z., Wang, L., Li, W., Zhang, H., Zhao, X.Y., Liu, L., Wang, Z., et al. (2012b). Direct reprogramming of Sertoli cells into multipotent neural stem cells by defined factors. Cell Res. *22*, 208–218.

Shu, J., Wu, C., Wu, Y., Li, Z., Shao, S., Zhao, W., Tang, X., Yang, H., Shen, L., Zuo, X., et al. (2013). Induction of pluripotency in mouse somatic cells with lineage specifiers. Cell *153*, 963–975.

Son, E.Y., Ichida, J.K., Wainger, B.J., Toma, J.S., Rafuse, V.F., Woolf, C.J., and Eggan, K. (2011). Conversion of mouse and human fibroblasts into functional spinal motor neurons. Cell Stem Cell *9*, 205–218.

Song, K., Nam, Y.J., Luo, X., Qi, X., Tan, W., Huang, G.N., Acharya, A., Smith, C.L., Tallquist, M.D., Neilson, E.G., et al. (2012). Heart repair by reprogramming non-myocytes with cardiac transcription factors. Nature *485*, 599–604.

Soufi, A., Donahue, G., and Zaret, K.S. (2012). Facilitators and impediments of the pluripotency reprogramming factors' initial engagement with the genome. Cell *151*, 994–1004.

Szabo, E., Rampalli, S., Risueño, R.M., Schnerch, A., Mitchell, R., Fiebig-Comyn, A., Levadoux-Martin, M., and Bhatia, M. (2010). Direct conversion of human fibroblasts to multilineage blood progenitors. Nature *468*, 521–526.

Tabar, V., and Studer, L. (2014). Pluripotent stem cells in regenerative medicine: challenges and recent progress. Nat. Rev. Genet. *15*, 82–92.

Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell *126*, 663–676.

Takeuchi, J.K., and Bruneau, B.G. (2009). Directed transdifferentiation of mouse mesoderm to heart tissue by defined factors. Nature *459*, 708–711.

Theunissen, T.W., and Jaenisch, R. (2014). Molecular control of induced pluripotency. Cell Stem Cell *14*, 720–734.

Thier, M., Wörsdörfer, P., Lakes, Y.B., Gorris, R., Herms, S., Opitz, T., Seiferling, D., Quandel, T., Hoffmann, P., Nöthen, M.M., et al. (2012). Direct conversion of fibroblasts into stably expandable neural stem cells. Cell Stem Cell *10*, 473–479.

Thorel, F., Népote, V., Avril, I., Kohno, K., Desgraz, R., Chera, S., and Herrera, P.L. (2010). Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. Nature *464*, 1149–1154.

Tiemann, U., Sgodda, M., Warlich, E., Ballmaier, M., Schöler, H.R., Schambach, A., and Cantz, T. (2011). Optimal reprogramming factor stoichiometry increases colony numbers and affects molecular characteristics of murine induced pluripotent stem cells. Cytometry A *79*, 426–435.

Torper, O., Pfisterer, U., Wolf, D.A., Pereira, M., Lau, S., Jakobsson, J., Björklund, A., Grealish, S., and Parmar, M. (2013). Generation of induced neurons via direct conversion in vivo. Proc. Natl. Acad. Sci. USA *110*, 7038–7043.

Tursun, B., Patel, T., Kratsios, P., and Hobert, O. (2011). Direct conversion of C. elegans germ cells into specific neuron types. Science *331*, 304–308.

Uhlenhaut, N.H., Jakob, S., Anlag, K., Eisenberger, T., Sekido, R., Kress, J., Treier, A.C., Klugmann, C., Klasen, C., Holter, N.I., et al. (2009). Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. Cell *139*, 1130–1142.

Vierbuchen, T., and Wernig, M. (2011). Direct lineage conversions: unnatural but useful? Nat. Biotechnol. *29*, 892–907.

Vierbuchen, T., Ostermeier, A., Pang, Z.P., Kokubu, Y., Südhof, T.C., and Wernig, M. (2010). Direct conversion of fibroblasts to functional neurons by defined factors. Nature *463*, 1035–1041.

Wada, R., Muraoka, N., Inagawa, K., Yamakawa, H., Miyamoto, K., Sadahiro, T., Umei, T., Kaneda, R., Suzuki, T., Kamiya, K., et al. (2013). Induction of human cardiomyocyte-like cells from fibroblasts by defined factors. Proc. Natl. Acad. Sci. USA *110*, 12667–12672.

Wang, L., Wang, L., Huang, W., Su, H., Xue, Y., Su, Z., Liao, B., Wang, H., Bao, X., Qin, D., et al. (2013). Generation of integration-free neural progenitor cells from cells in human urine. Nat. Methods *10*, 84–89.

Wang, L., Liu, Z., Yin, C., Asfour, H., Chen, O.M., Li, Y., Bursac, N., Liu, J., and Qian, L. (2014). Stoichiometry of Gata4, Mef2c, and Tbx5 influences the efficiency and quality of induced cardiac myocyte reprogramming. Circ. Res. *116*, 237–244.

Wapinski, O.L., Vierbuchen, T., Qu, K., Lee, Q.Y., Chanda, S., Fuentes, D.R., Giresi, P.G., Ng, Y.H., Marro, S., Neff, N.F., et al. (2013). Hierarchical mechanisms for direct reprogramming of fibroblasts to neurons. Cell *155*, 621–635.

Xie, H., Ye, M., Feng, R., and Graf, T. (2004). Stepwise reprogramming of B cells into macrophages. Cell *117*, 663–676.

Xu, H.S., Yi, B.A., and Chien, K.R. (2012). In vivo reprogramming for heart disease. Cell Res. *22*, 1521–1523.

Xue, Y., Ouyang, K., Huang, J., Zhou, Y., Ouyang, H., Li, H., Wang, G., Wu, Q., Wei, C., Bi, Y., et al. (2013). Direct conversion of fibroblasts to neurons by reprogramming PTB-regulated microRNA circuits. Cell *152*, 82–96.

Yamaguchi, S., Hirano, K., Nagata, S., and Tada, T. (2011). Sox2 expression effects on direct reprogramming efficiency as determined by alternative somatic cell fate. Stem Cell Res. (Amst.) *6*, 177–186.

Yan, L., Yang, M., Guo, H., Yang, L., Wu, J., Li, R., Liu, P., Lian, Y., Zheng, X., Yan, J., et al. (2013). Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. Nat. Struct. Mol. Biol. *20*, 1131–1139.

Yang, N., Zuchero, J.B., Ahlenius, H., Marro, S., Ng, Y.H., Vierbuchen, T., Hawkins, J.S., Geissler, R., Barres, B.A., and Wernig, M. (2013). Generation of oligodendroglial cells by direct lineage conversion. Nat. Biotechnol. *31*, 434–439.

Yang, R., Zheng, Y., Li, L., Liu, S., Burrows, M., Wei, Z., Nace, A., Herlyn, M., Cui, R., Guo, W., et al. (2014). Direct conversion of mouse and human fibroblasts to functional melanocytes by defined factors. Nat. Commun. *5*, 5807.

Yechoor, V., Liu, V., Espiritu, C., Paul, A., Oka, K., Kojima, H., and Chan, L. (2009). Neurogenin3 is sufficient for transdetermination of hepatic progenitor cells into neo-islets in vivo but not transdifferentiation of hepatocytes. Dev. Cell *16*, 358–373.

Yoo, A.S., Sun, A.X., Li, L., Shcheglovitov, A., Portmann, T., Li, Y., Lee-Messer, C., Dolmetsch, R.E., Tsien, R.W., and Crabtree, G.R. (2011). MicroRNA-mediated conversion of human fibroblasts to neurons. Nature *476*, 228–231.

Yu, B., He, Z.Y., You, P., Han, Q.W., Xiang, D., Chen, F., Wang, M.J., Liu, C.C., Lin, X.W., Borjigin, U., et al. (2013). Reprogramming fibroblasts into bipotential hepatic stem cells by defined factors. Cell Stem Cell *13*, 328–340.

Yuan, Y., Hartland, K., Boskovic, Z., Wang, Y., Walpita, D., Lysy, P.A., Zhong, C., Young, D.W., Kim, Y.K., Tolliday, N.J., et al. (2013). A small-molecule inducer of PDX1 expression identified by high-throughput screening. Chem. Biol. *20*, 1513–1522.

Zaret, K.S., and Carroll, J.S. (2011). Pioneer transcription factors: establishing competence for gene expression. Genes Dev. *25*, 2227–2241.

Zhang, K., Liu, G.H., Yi, F., Montserrat, N., Hishida, T., Rodriguez Esteban, C., and Izpisua Belmonte, J.C. (2013). Direct conversion of human fibroblasts into retinal pigment epithelium-like cells by defined factors. Protein Cell *5*, 48–58.

Zhao, D.X., Chen, S., Duo, S.G., Xiang, C.G., Jia, J., Guo, M.N., Lai, W., Lu, S.C., and Deng, H.K. (2013). Promotion of the efficient metabolic maturation of human pluripotent stem cell-derived hepatocytes by correcting specification defects. Cell Res. *23*, 157–161.

Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J., and Melton, D.A. (2008). In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature *455*, 627–632.

Zhu, S., Ambasudhan, R., Sun, W., Kim, H.J., Talantova, M., Wang, X., Zhang, M., Zhang, Y., Laurent, T., Parker, J., et al. (2014a). Small molecules enable OCT4-mediated direct reprogramming into expandable human neural stem cells. Cell Res. *24*, 126–129.

Zhu, S., Rezvani, M., Harbell, J., Mattis, A.N., Wolfe, A.R., Benet, L.Z., Willenbring, H., and Ding, S. (2014b). Mouse liver repopulation with hepatocytes generated from human fibroblasts. Nature *508*, 93–97.

Zuryn, S., Ahier, A., Portoso, M., White, E.R., Morin, M.C., Margueron, R., and Jarriault, S. (2014). Transdifferentiation. Sequential histone-modifying activities determine the robustness of transdifferentiation. Science *345*, 826–829.