Pluripotent stem cells can self-renew indefinitely and undergo differentiation to produce all types of cells in adult bodies. They could be used to generate various cells or even tissues/organs for transplantation. Patients with injuries, degenerative disease, aging, or cancers would benefit from the realization of stem cell–based regenerative medicine. To avoid rejection by the patient’s immune system, cell-based therapies preferably use immune-matched donor cells, or the patient’s
own cells, which could be derived from their own pluripotent stem cells.

**Reprogramming to Pluripotency**
Various methods have been developed to reprogram cells to a pluripotent state. In the 1960s, somatic cell nuclear transfer (SCNT) was reported to erase lineage-specific signatures in the nuclei of a somatic cell and reprogram it to a totipotent state.  

So far, SCNT approach has been successfully performed in mouse and some other species, but not yet reported in human. Fusion of somatic cells with pluripotent cells was another method proven to enable reprogramming somatic cells to pluripotent cells; however, the utility of this method is limited because the resultant cells are tetraploid. Both methods take advantage of cellular materials to establish pluripotency in somatic cells; SCNT uses material from oocytes, and cell fusion uses material from pluripotent cells.

Although SCNT and cell fusion–induced reprogramming are deterministic and relatively fast and efficient, significant technical challenges (as well as ethical challenges in the case of SCNT) remain before they can be practical. In addition, they entail complex mixtures of known and undefined factors from oocytes or pluripotent cells to trigger reprogramming, making mechanistic studies more challenging.

These barriers might be avoided by a new strategy in which mammalian somatic cells are reprogrammed to induced pluripotent stem cells (iPSCs) by ectopic expression of the pluripotent transcription factors (TFs) Oct4, Sox2, Klf4, and c-Myc (or Nanog and Lin28 instead of Klf4 and c-Myc). In practice, combined with efficient differentiation strategies, iPSCs would be valuable not only to derive functional cells for transplantation but also to establish patient-specific disease models for drug discovery and development. Similar to the capability of cell-type-specific TFs to maintain cell identity by binding to specific DNA sequences across the genome and achieving additional sequence-binding specificity and transcriptional/epigenetic regulation by forming complexes with coregulatory factors, exogenous iPSC TFs (TFs overexpressed in generation of iPSCs) cooperatively remodel chromatin to activate expression of genes in the pluripotency network and to suppress expression of genes that promote differentiation. In addition, through both co-occupancy with downstream effectors of various signaling pathways and recruitment of diverse epigenetic enzymes over the whole genome, specific chromosomal binding patterns of exogenous iPSC TFs during the reprogramming process contribute to the establishment of iPSC-specific signal transduction, transcriptional circuitry, and epigenetic pattern (Figure 1).

Although iPSC reprogramming is technically simpler than SCNT and cell fusion, it only induces a stochastic and non-specific reprogramming process and is therefore less efficient and slower than SCNT and cell fusion. This difference reflects the possibility that iPSC TFs are core components, but not the complete machinery functional in efficient and specific reprogramming induced by both SCNT and cell fusion. Besides, iPSCs generated by conventional methods raise concerns about their safety (eg, immunogenicity and the risk of tumorigenesis) for clinical applications, as they use virus-mediated gene delivery that results in genomic integration of exogenous sequence and enforced expression of oncogenes.

To increase efficiency, accelerate kinetics, and reduce safety concerns, many improvements in methodology have been achieved by different groups. Several specific cell types were shown to enable reprogramming with higher efficiency and less number of exogenous iPSC TFs. However, nearly all of them rely on overexpression of exogenous iPSC TFs and extra manipulations (eg, administration of cytokines or small molecules) to get reprogrammed efficiently and rapidly. In addition to reprogramming using different starting cell types, methods using virus-free, removable PiggyBac transposons, minicircle systems, or episomal systems have been developed. Despite their success in generating iPSCs, often without a genetic footprint, the use of DNA constructs leaves the possibility of genomic integration of exogenous sequence. Other attempts to generate iPSCs by nonintegrating virus-mediated gene delivery cannot preclude the safety concerns raised by using viruses. Although recombinant proteins or synthetic mRNAs can produce iPSCs, the protocols are costly and technically challenging. Recently, microRNA was used to generate reprogrammed iPSCs, but the practical use and robustness of this approach are uncertain.

In addition, generation of iPSC with small molecules alone is being attempted. This promising strategy might eliminate many of the drawbacks (eg, the risk of tumorigenesis from genomic integration of exogenous sequence or overexpression of oncogenes) of conventional and other improved iPSC reprogramming methods. Although the final outcome of iPSC reprogramming induced by any method is the establishment of pluripotency-associated gene expression profile and epigenetic pattern, the small molecule approach would use a different process/mechanism from other methods to launch reprogramming process. In details, in other methods, exogenously introduced and pluripotency-associated elements (eg, TFs) trigger iPSC reprogramming by directly participating in and directing pluripotency-specific chromatin remodeling in somatic cells, whereas the small molecule approach indirectly initiates iPSC reprogramming by mediating endogenous, nonpluripotency-specific components in somatic cells (Figure 1). Therefore, at this point, successful induction of iPSCs by small molecules would fundamentally change the concept of iPSC reprogramming. Besides, all small molecules identified during development of this method would be possible candidates to further improve iPSC reprogramming and investigate the underlying mechanisms of this process. To date, many small molecules have been identified to modulate the induction of iPSCs, in both functionally replacing some exogenous iPSC TFs and significantly improving the efficiency and quality of iPSC reprogramming (Table).

**Transdifferentiation**
There are 2 promising strategies to regenerate tissue-specific cell types. In one, pluripotent cells are differentiated into

---

**Nonstandard Abbreviations and Acronyms**

- ESC: embryonic stem cell
- iPSC: induced pluripotent stem cell
- MEF: mouse embryonic fibroblast
- SCNT: somatic cell nuclear transfer
- TF: transcription factor

---
various types of somatic cells. In the other, conventionally known as transdifferentiation, somatic cells are directly reprogrammed to another type of cells. Both approaches could be useful in the field of regenerative medicine and for disease modeling. Transdifferentiation has the advantage of avoiding the use of iPSCs to derive patient-specific cells, making the process faster and more efficient, reducing the risk of pluripotency-associated tumorigenesis and probably avoiding
immunogenicity identified recently in iPSCs. In fact, many classic studies of transdifferentiation were thoroughly investigated before or around the time of discovery of iPSCs. For instance, ectopic expression of MyoD induced conversion of fibroblasts to myoblasts, expression of GATA1 or PU1 promoted the reciprocal transition between myeloid cells and megakaryocyte/erythroid cells, expression of C/EBPα or C/EBPβ converted pre-B or pre-T cells to macrophages, and

Table. Small Molecules Identified in Induced Pluripotent Stem Cell Research

<table>
<thead>
<tr>
<th>Name and Structure</th>
<th>Target(s)/Function</th>
<th>Effect(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG108</td>
<td>DNA methyltransferase inhibitor</td>
<td>In the presence of BIX-01294, improves reprogramming of MEFs induced by Oct4 and Klf4.</td>
</tr>
<tr>
<td>Bayk8644 (BayK)</td>
<td>L-calcium channel agonist</td>
<td>In the presence of BIX-01294, improves reprogramming of MEFs induced by Oct4 and Klf4.</td>
</tr>
<tr>
<td>Parnate</td>
<td>Lysine-specific demethylase 1 inhibitor</td>
<td>In combination with CHIR99021, enables reprogramming of human keratinocytes induced by Oct4 and Klf4.</td>
</tr>
<tr>
<td>5-Azacytidine</td>
<td>DNA methyltransferase inhibitor</td>
<td>Improves MEF reprogramming efficiency with four iPSC TFs or under c-Myc-free condition. Facilitates erasure of epigenetic memory retained in established iPSCs.</td>
</tr>
<tr>
<td>Suberoylanilide hydroxamic acid (SAHA)</td>
<td>HDAC inhibitor</td>
<td>Improves MEF reprogramming efficiency with 4 iPSC TFs.</td>
</tr>
<tr>
<td>Trichostatin A (TSA)</td>
<td>HDAC inhibitor</td>
<td>Improves MEF reprogramming efficiency with four iPSC TFs. Facilitates erasure of epigenetic memory retained in established iPSCs.</td>
</tr>
<tr>
<td>Valproic acid (VPA)</td>
<td>HDAC inhibitor</td>
<td>Improves MEF reprogramming efficiency with four iPSC TFs or under c-Myc-free condition. Enables reprogramming of human fibroblasts induced by Oct4 and Sox2.</td>
</tr>
</tbody>
</table>

(continued)
Table.  (Continued)

<table>
<thead>
<tr>
<th>Name and Structure</th>
<th>Target(s)/Function</th>
<th>Effect(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenpaullone</td>
<td>Glycogen synthase kinase 3β and cyclin-dependent kinases inhibitor</td>
<td>Functionally replaces Klf4 in reprogramming of MEFs in the presence of Oct4, Sox2, and c-Myc.</td>
</tr>
<tr>
<td>SB431542</td>
<td>TGFβ receptor inhibitor</td>
<td>Enhances MEF reprogramming efficiency in the absence of c-Myc. Enhances and accelerates reprogramming of human somatic cells. Functionally replaces Sox2 in MEF reprogramming.</td>
</tr>
<tr>
<td>PD0325901</td>
<td>MEK inhibitor</td>
<td>Enhances and accelerates reprogramming of somatic cells.</td>
</tr>
<tr>
<td>Thiazovivin</td>
<td>ROCK inhibitor</td>
<td>Enhances and accelerates reprogramming of human somatic cells.</td>
</tr>
<tr>
<td>A-83-01</td>
<td>TGFβ receptor inhibitor</td>
<td>Enhances MEF reprogramming. In combination with AMI-5, enables reprogramming of MEFs transduced with Oct4 only.</td>
</tr>
</tbody>
</table>

(continued)
### Table. (Continued)

<table>
<thead>
<tr>
<th>Name and Structure</th>
<th>Target(s)/Function</th>
<th>Effect(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMI-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="AMI-5 structure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein arginine methyltransferase inhibitor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In combination with A-83-01, enables reprogramming of MEFs transduced with Oct4 only.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-Bromoadenosine 3,5'-cyclic monophosphate (8-Br-cAMP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="8-Br-cAMP structure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein kinase A activator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improves the reprogramming efficiency of human neonatal foreskin fibroblasts transduced with all 4 iPSC TFs.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dasatinib</td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Dasatinib structure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Src family kinases Inhibitor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functionally replaces Sox2 in MEF reprogramming.75,76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="PP1 structure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Src family kinases inhibitor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functionally replaces Sox2 in MEF reprogramming.75,76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iPYrazine (iPY)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="iPYrazine structure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Src family kinases inhibitor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functionally replaces Sox2 in MEF reprogramming.75,76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS48</td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="PS48 structure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3'-Phosphoinositide-dependent kinase 1 activator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In combination with A-83-01, PD0325901 and sodium butyrate, enables reprogramming of human somatic cells transduced with Oct4 only.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium butyrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Sodium butyrate structure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDAC inhibitor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In combination with A-83-01, PD0325901, and PS48, enables reprogramming of human somatic cells transduced with Oct4 only.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Vitamin C structure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioxidant and enzyme cofactor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Promotes iPSC generation from both mouse and human somatic cells.30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
expression of C/EBPβ provoked conversion from pancreatic cells to hepatocytes. However, most of these transitions were restricted to a relatively narrow lineage (eg, hematopoietic, mesenchymal, or foregut endodermal system) and were induced by overexpression of a single TF. Success in generating iPSCs suggests constitutive expression of several TFs together might more efficiently induce transdifferentiation between less related cell types. This hypothesis has been verified. Many transdifferentiations induced by a set of lineage-specific TFs have been performed in vitro and in vivo. In addition, a new concept, iPSC TF-based transdifferentiation, was recently proposed and demonstrated: transient overexpression of iPSC TFs in conjunction with cell-type–specific signals can reprogram somatic cells into diverse lineage-specific cells without going through the pluripotent state. Furthermore, compared with conventional transdifferentiation, this new method has many advantages, such as the use of universal TF system and the ability to generate a multipotent progenitor population.

Here, we will categorize all small molecules identified in iPSC research, based on their function in target cells, and review them separately. Then we will review iPSC TF–induced transdifferentiation and its use to transdifferentiate somatic cells into various lineage-specific cells, especially cardiovascular cells.

Epigenetic Modifiers
During reprogramming, cells not only undergo transcriptional changes but also exhibit epigenetic changes in DNA methylation and histone modifications. These changes convert the epigenetic pattern of somatic cells to an embryonic stem cell (ESC)–like state. Several small molecules that target enzymes involved in epigenetic modifications increase the efficiency of cellular reprogramming and sometimes can even functionally replace ectopic expression of certain TFs.

Dramatic changes in the histone methylation pattern are key features of iPSC reprogramming. Thus, reprogramming would be affected by small molecules that target enzymes involved in histone methylation or demethylation. BIX-01294 (BIX), an inhibitor of histone H3K9 mono- and dimethyltransferase G9a, enables reprogramming by ectopic expression of Oct4 and Klf4 in somatic cells. Moreover, after treatment of neural progenitor cells with BIX-01294 or treatment of mouse embryonic fibroblasts (MEFs) with BIX-01294 combined with a DNA methyltransferase inhibitor RG108 or an L-calcium channel agonist Bayk8644, reprogramming with only 2 TFs (ie, Oct4 and Klf4) was as efficient as reprogramming with 4 TFs. Consistently, G9a-mediated H3K9 methylation is necessary for heterochromatinization and silencing of key pluripotency genes, such as Oct4 and Rex1, during early embryogenesis. Parnate is an inhibitor of lysine-specific demethylase 1 (LSD1)–mediated H3K4 demethylation, shown to globally increase H3K4 methylation, as well as transcriptional derepression of lysine-specific demethylase 1 target gene Oct4 in P19 embryonal carcinoma cells. In line with this observation, Parnate enabled 2 factors
(Oct4 and Klf4) to induce conversion of human keratinocytes to iPSCs, when combined with CHIR99021 (an inhibitor of glycogen synthase kinase 3β).

Because inhibition of histone deacetylation and DNA methylation improves the reprogramming efficiency of SCNT, it was hypothesized that such an approach might also aid in the establishment of iPSCs. As predicted, a DNA methyltransferase inhibitor, 5-azacytidine, or 3 histone deacetylase inhibitors (suberoylanilide hydroxamic acid, trichostatin A, and valproic acid) improves reprogramming efficiency after transduction of the 4 iPSC TFs in MEFs. In addition, treatment with 5-azacytidine or valproic acid increases reprogramming efficiency under c-Myc-free conditions. Further mechanistic studies showed that 5-azacytidine induced a rapid and stable transition of a fully reprogrammed iPSC state from a partially reprogrammed state with DNA hypermethylation at pluripotency genes, and administration of valproic acid in MEFs upregulated some ESC-specific genes meanwhile downregulated some MEF-specific genes. Moreover, valproic acid enables reprogramming of human fibroblasts transduced with only Oct4 and Sox2 and has been used to efficiently generate recombinant protein–induced pluripotent stem cells.

Although mouse iPSC clones that are functionally identical to ESCs can be generated, subtle differences in gene expression and epigenetic patterns between iPSCs and ESCs could exist in some other iPSC clones. Such differences could reflect residual expression and epigenetic pattern of original cell-type–specific genes and gained genetic and epigenetic changes, contributed by incomplete epigenetic reprogramming or induced changes imposed by the reprogramming process. To improve on such incomplete reprogramming, epigenetic memory can be largely erased by treating established iPSCs with 5-azacytidine and trichostatin A. On the other hand, such epigenetic memory may favor differentiation of iPSCs toward lineages related to its original cell type.

In summary, appropriate application of small molecules that function as epigenetic modifiers, either individually or in combination, significantly increases iPSC reprogramming efficiency. Furthermore, administration of epigenetic modifiers can largely diminish epigenetic memory retained in iPSCs, and thereby improve the quality of reprogrammed iPSCs.

Signaling Modulators
Consistent with the notion that signal transduction pathways mediated by extrinsic factors and intrinsic transcriptional network cooperate to maintain self-renewal and pluripotency of ESCs, signal transduction pathways and TFs act coordinately to reprogram somatic cells to iPSCs. Therefore, small molecules that target signaling pathways would modulate reprogramming. Some such small molecules have been identified that increase reprogramming efficiency and can functionally replace some TFs in iPSC reprogramming.

Wnt signaling is important for maintaining the pluripotency of ESCs and for self-renewal of adult stem cells in multiple tissues. In ESCs, TCF3, a downstream effector of the Wnt signaling pathway, co-occupies genome-wide loci with master pluripotency regulators, such as Oct4 and Nanog, and acts as a transcriptional repressor for targeted genes, competing activity of these master pluripotency regulators. β-Catenin, which is stabilized by activation of the Wnt signaling pathway, directly interacts with TCF3 and reduces inhibitory effect of TCF3 on pluripotency. Therefore, the Wnt signaling pathway is regarded as an integral component of the core transcriptional circuitry in ESCs. Indeed, β-catenin was involved in initial study of iPSC reprogramming, and deletion of TCF3 could strikingly and rapidly enhance the efficiency of neural progenitor cell reprogramming. Consistently, Wnt3a enhanced the reprogramming of MEFs to pluripotency in the absence of c-Myc; CHIR99021, a glycogen synthase kinase 3β inhibitor that activates Wnt signaling, significantly improved reprogramming efficiency in MEFs in the absence of Sox2 and c-Myc.

In the same study, treatment with CHIR99021 and Parnate converted human keratinocytes to iPSCs on ectopic expression of Oct4 and Klf4. Another glycogen synthase kinase 3β inhibitor, kenpaullone, which also inhibits other kinases, could replace Klf4 in reprogramming of MEFs in the presence of Oct4, Sox2, and c-Myc. Interestingly, the role of kenpaullone in this process could not be replaced by CHIR99021, and the mechanism is unknown.

During reprogramming, mesenchymal-type fibroblasts undergo dramatic epithelial-like morphological changes and correlated gene expression changes, such as upregulation of E-cadherin (which is highly expressed in pluripotent cells) and concomitant downregulation of Snail. This so-called mesenchymal-to–epithelial transition inevitably occurs during successful reprogramming of cells. Therefore, small molecules that facilitate the mesenchymal-to-epithelial transition process were hypothesized to enhance reprogramming. Indeed, small molecules that target 3 known mesenchymal-to-epithelial transition mechanisms (for repression of epithelial phenotype with upregulation and stabilization of E-cadherin), including inhibition of TGFβ receptor (by SB431542), MEK (by PD0325901), or ROCK (by thiazovivin), individually or in combination significantly enhanced reprogramming of human somatic cells and increased reprogramming speed. This mesenchymal-to-epithelial transition mechanism was further characterized in 3 subsequent studies, where additional small molecules that inhibit the TGFβ pathway or upregulate E-cadherin were used.

More recently, another TGFβ receptor inhibitor, A-83-01, combined with a protein arginine methyltransferase inhibitor, AMI-5, enabled reprogramming of MEFs transduced with Oct4 alone.

A few other small molecules that affect many other signaling pathways also facilitate iPSC reprogramming. A cAMP analog, 8-bromoadenosine 3′,5′-cyclic monophosphate, improved the reprogramming efficiency of human neonatal foreskin fibroblasts transduced with all 4 iPSC TFs. Inhibitors of Src family kinases, including Dasatinib, PP1, and IPyrazine, sufficiently supported reprogramming of MEFs in the absence of exogenous Sox2, as efficiently as TGFβ inhibitors. All these observations are consistent with previous reports in which the PKA pathway and Src family kinases were shown to influence ESC self-renewal and differentiation.

In summary, small molecule modulators of signaling pathways, individually or in combination, and sometimes even together with epigenetic modifiers, induce reprogramming with higher efficiency and fewer exogenous TFs, by affecting the
integrated cellular network consisting of both signaling pathways and transcriptional circuitry.

**Cell Senescence Alleviators**

Cell senescence is typically seen during cellular reprogramming and is considered one of the barriers of reprogramming that account for the slow kinetics and low efficiency of this process.80 Indeed, the early stage of iPSC reprogramming entails a stress response with characteristics of cell senescence, including upregulated expression of p53, p21cip, and p16ink4a.81,82 Furthermore, downregulating the expression of any of these genes increased the efficiency and speed of iPSC reprogramming.83-87 A natural antioxidant, vitamin C, promoted iPSC generation from both mouse and human somatic cells through indirect reduction of p53 and p21cip expression and partial alleviation of cell senescence.88

**Metabolism Regulators**

Differentiated adult somatic cells typically use mitochondrial oxidative phosphorylation for cell growth, whereas pluripotent stem cells mainly use glycolytic metabolism.89-91 In rapidly proliferating pluripotent stem cells, glycolytic metabolism more effectively produces various macromolecules necessary for cell proliferation and to improve the efficiency of iPSC reprogramming.90,91 This metabolic reprogramming is further supported by the ability of hypoxia-inducible factor 1α and c-Myc to promote glycolytic metabolism and to improve the efficiency of iPSC reprogramming.92,93 Consistently, a recent study showed PS48, a small molecule activator of 3′-phosphoinositide-dependent kinase 1, combined with A-83-01, PD0325901, and sodium butyrate (a histone deacetylase inhibitor) enabled reprogramming of human mitochondrial oxidative phosphorylation to glycolytic metabolism more effectively produces various macromolecular precursors to meet the metabolic/energy demand and generates fewer reactive oxygen species, which induce oxidative stress.89,91 Therefore, transition from mitochondrial oxidative phosphorylation to glycolytic metabolism would be a crucial step in iPSC reprogramming.92,93 This metabolic reprogramming is further supported by the ability of hypoxia-inducible factor 1α and c-Myc to promote glycolytic metabolism and to improve the efficiency of iPSC reprogramming.92,93

Consistently, a recent study showed PS48, a small molecule activator of 3′-phosphoinositide-dependent kinase 1, combined with A-83-01, PD0325901, and sodium butyrate (a histone deacetylase inhibitor) enabled reprogramming of human adult keratinocytes, umbilical vein endothelial cells, or amniotic fluid–derived cells transduced with only Oct4 and c-Myc to promote glycolytic metabolism and to improve the efficiency of iPSC reprogramming.92,93 Remarkably, this study also revealed iPSC reprogramming efficiency was significantly increased by several other small molecules that enhance glycolytic metabolism and depress mitochondrial oxidative phosphorylation, including 2,4-dinitrophenol (an oxidative phosphorylation uncoupler), fructose 2,6-bisphosphate (an activator of phosphofructokinase 1), N-oxalylglycine (an inhibitor of pyruvate dehydrogenase), and quercetin (an activator of hypoxia-inducible factor pathway).92 In a word, all evidence associated with these small molecules confirm the notion of metabolic reprogramming.

**Transdifferentiation**

Built on iPSC reprogramming strategy and previous studies on cellular transdifferentiation restricted within several lineages, functional neurons, cardiomyocytes, hepatocytes, or macrophage-like cells can be converted from fibroblasts by ectopic expression of multiple lineage-specific TFs or microRNAs.90-93 Furthermore, transdifferentiation has also been induced with lineage-specific TFs in vivo.94,95 However, transdifferentiated cells generated by ectopic expression of lineage-specific TFs were mostly one type of terminally differentiated cells. Therefore, conventional transdifferentiation typically would not allow isolation, expansion, and characterization of the reprogrammed cells, all of which are prerequisite for clinical application. In a word, the restricted proliferative capacity and limited cell type diversity of these transdifferentiated cells may substantially compromise their potential application in regenerative therapy.

Recently, a new paradigm of transdifferentiation was devised to generate various lineage-specific precursor cells by combining transient overexpression of the iPSC TFs and treatment with soluble signaling molecules. The basis of this universal transdifferentiation strategy is that conventional iPSC reprogramming proceeds as a slow, step-wise, and nondeterminative process that gives rise to iPSCs only at the late stage and with low efficiency and to populations of epigenetically unstable/plastic cells at early and intermediate stages. In addition, generating iPSCs requires an extended period (eg, 8–12 days) of iPSC TF expression and a specific signaling environment (eg, leukemia inhibitory factor). These observations suggest that temporal iPSC TF expression combined with different signaling environments (eg, growth factors/cytokines, and small molecule modulators of signaling pathways) would dictate reprogrammed cell fate. This would be consistent with the notion that cell-type-specific TFs direct different cell fates cooperatively with interacted signaling downstream TFs. Indeed, this concept and strategy were demonstrated by direct conversion of fibroblasts to cardiac,93 neural,94 or definitive endoderm (unpublished data) precursor cells using transient expression of iPSC TFs (eg, 4 days), followed by treatment with BMP4, FGF4, or Activin-A, respectively. (Figure 2) Compared with conventional transdifferentiation, in which different cell specifications are determined by ectopic expression of different sets of lineage-specific TFs, all iPSC TF-based transdifferentiations share the same TFs in the initial step: transient overexpression of iPSC TFs. Such transient gene expression might be more easily replaced with safer and more convenient methods without genetic modifications. Next, we will separately review iPSC TF-induced transdifferentiation toward different lineages.

**iPSC TF-Based Transdifferentiation of Fibroblasts Into Cardiac Cells**

The mammalian heart lacks significant regenerative capacity, hence transplantation of autologous cardiac cells generated in vitro is considered a possible therapy for heart disease. In a previous report, 3 cardiac TFs, Gata4, Mef2c, and Tbx5, enabled transdifferentiation of mouse postnatal cardiac or dermal fibroblasts directly into cardiomyocyte-like cells.95 However, it took several weeks to generate beating cardiomyocytes, and the efficiency was very low. As an alternative method, temporal expression of iPSC TFs (Oct4/Sox2/Klf4/ c-Myc) in mouse fibroblasts for only 4 to 6 days with a JAK inhibitor and without leukemia inhibitory factor, followed by treatment with BMP4 in a chemically defined medium for additional 5 days, resulted in a large number of spontaneously contracting cardiomyocytes.96 This process was further characterized...
as a direct transdifferentiation, during which no pluripotent intermediates arose. Furthermore, an inverse relationship was demonstrated between induction of cardiomyocytes and generation of iPSCs, which could explain why cardiogenic transdifferentiation was facilitated by small molecule inhibition of pluripotency but was impaired by prolonged overexpression of iPSC TFs.94

Compared with the cardiac TF–induced transdifferentiation, cardiogenic transdifferentiation triggered by transient expression of iPSC TFs followed by treatment with BMP4 is much faster (first spontaneous contractions beginning after 11 days versus 4–5 weeks) and more efficient.31,94 Moreover, multipotent cardiovascular precursor cells arose at an intermediate stage, as suggested by the pattern of gene expression during iPSC TF-induced transdifferentiation.94 If such cells could be isolated and expanded in vitro, they would eventually be a renewable source for unlimited amounts of cardiomyocytes and other terminally differentiated cardiovascular cells as well. Therefore, relative to cardiac TF–induced terminally differentiated cardiomyocytes, which are restricted in cell type and limited in capacity to propagate after transplantation in vivo,93 these multipotent cardiovascular precursor cells may be a more versatile cell source for modeling cardiovascular disease and for cell-based therapy.

For ultimate clinical applications, it would be highly desirable to develop a more robust condition for human systems without any genetic modifications and safety concerns. Knowledge and techniques gained from the development of iPSC reprogramming might be especially helpful to improve this new method of cardiogenic transdifferentiation.

**iPSC TF-Based Transdifferentiation of Fibroblasts Toward Other Lineages**

In addition to cardiac cells, other lineage-specific precursor cells, such as neural and definitive endoderm precursor cells, have also been generated from mouse and human fibroblasts by the same paradigm with different signaling molecules.95 In these studies, transient expression (4–6 days) of iPSC TFs in fibroblasts was the initial step shared by all. Another shared step was treatment with a JAK inhibitor to suppress the leukemia inhibitory factor-STAT3 pathway during iPSC reprogramming, which would prevent establishment of pluripotency in reprogrammed cells and facilitate the generation of developmentally plastic intermediate cells. The epigenetically activated cells were treated with FGF4 to generate neural precursor cells or with Activin-A to generate definitive endoderm precursor cells. Importantly, the efficiently and fast converted neural precursor cells could be expanded for serial passages and then differentiated into mature and subtype-specific neuronal cells and glial cells.95 In addition, human fibroblasts have been directly converted to multipotent blood progenitors by prolonged ectopic expression of Oct4 and treatment with IGFII, bFGF, Flt3L, and SCF.96

In summary, cardiovascular cells and other lineage-specific precursor cells can be directly converted from iPSC TF-induced transdifferentiation, which implies that such strategy would provide a general platform to induce a broad range of cells for various applications.

**Perspectives**

The stem cell field has embarked on exciting discoveries that both iPSCs and lineage-specific cells can be reprogrammed from somatic cells by ectopic expression of iPSC TFs. Because of the close relationship between these 2 types of reprogramming, they share some technical challenges and safety considerations that need to be addressed before their clinical applications. To date, several strategies have overcome these hurdles with respect to iPSC reprogramming, and some may also work well in iPSC TF–based transdifferentiation. As reviewed above, regarding iPSC reprogramming, small molecules are valuable not only to significantly promote cellular reprogramming and functionally substitute ectopic expression
of TFs but also to provide insights into molecular mechanism underlying this process. Although pleiotropy of small molecules may result in some side effects compromising the desired reprogramming process, appropriate employment of small molecule combinations would largely diminish these side effects and potentially have synergistic effects on reprogramming. In the future, complete small molecule–based reprogramming will fundamentally change the reprogramming paradigm through a mechanism that involves activation of endogenous TFs by small molecules rather than by exogenously provided reprogramming TFs. Moreover, the success of small molecule–based transdifferentiation would greatly reduce safety concerns in the clinical application of reprogrammed cells by avoiding issues caused both by the generation of pluripotent cells (eg, the risk of pluripotency-associated tumorigenesis) and by the introduction of exogenous TFs. Therefore, better understanding of these reprogramming processes and further development of these small molecules may ultimately be useful for in vivo stem cell biology and therapy.

Acknowledgments
We thank Saiyong Zhu, Mingliang Zhang, Baoming Nie, Yu Zhang, Chen Yu, and other members in Ding laboratory for their constructive discussions, Stephen Ordway and Gary Howard for editing the manuscript, and the Gladstone Graphics Department for assistance on figures.

Sources of Funding
Sheng Ding is supported by funding from National Institute of Child Health and Human Development, National Heart, Lung, and Blood Institute, National Eye Institute, and National Institute of Mental Health/National Institutes of Health, California Institute for Regenerative Medicine, Prostate Cancer Foundation, and the Gladstone Institute.

Disclosures
None.

References


Progress in the Reprogramming of Somatic Cells
Tianhua Ma, Min Xie, Timothy Laurent and Sheng Ding

Circ Res. 2013;112:562-574
doi: 10.1161/CIRCRESAHA.111.249235
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/112/3/562

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/