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REVIEW

Small molecules for reprogramming and transdifferentiation

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Abstract Pluripotency reprogramming and transdifferentiation induced by transcription factors can generate induced pluripotent stem cells, adult stem cells or specialized cells. However, the induction efficiency and the reintroduction of exogenous genes limit their translation into clinical applications. Small molecules that target signaling pathways, epigenetic modifications, or metabolic processes can regulate cell development, cell fate, and function. In the recent decade, small molecules have been widely used in reprogramming and transdifferentiation fields, which can promote the induction efficiency, replace exogenous genes, or even induce cell fate conversion alone. Small molecules are expected as novel approaches to generate new cells from somatic cells in vitro and in vivo. Here, we will discuss the recent progress, new insights, and future challenges about the use of small molecules in cell fate conversion.

Keywords Adult cells · Chemical compound · Direct conversion · Tissue repair · Regeneration

Introduction

Somatic or adult cells are highly specialized and stable in normal settings. However, the somatic state can be broken under certain conditions and become pluripotent stem cells or another cell type. In 1958, reprogramming somatic cells to pluripotent stem cell was initially achieved by somatic cell nuclear transfer (SCNT) technology, in which the nucleus of somatic cells was transferred into the cytoplasm of the enucleated oocyte [1]. In 2006, Yamanaka et al. reintroduced four pluripotency transcription factors Oct4 (also known as POU5F1), SRY-box2 (Sox2), Krüpple-like factor 4 (Klf4), and c-Myc (collectively called OSKM) into mouse fibroblasts by viral vectors and produced embryonic stem cell (ESC)-like cells, namely induced pluripotent stem cells (iPSCs) [2]. iPSCs are able to differentiate any cell types, without ethical issues faced by ESCs. Thus, the discovery of iPSCs is a milestone for stem cell research and regenerative medicine. Yamanaka's study proposes a paradigm that reintroducing appropriate transcription factors can change the somatic cell fate and make them go into a new cell gene grogram. In agreement, forced expression of lineage-specific transcription factors have converted somatic cells (e.g., fibroblasts) into another lineage cell, such as cardiac cells [3, 4], neural cells [5, 6], hepatic cells [7] and so on (Fig. 3). The transdifferentiation process does not pass the pluripotent intermediate, and therefore, avoids the risk of tumor formation [3, 8–10]. Nevertheless, the genetic delivery of exogenous genes has some safety concerns such as genetic mutations and gene insertions.

Currently, small chemical compounds or small molecules have been emerging to play important roles in reprogramming and transdifferentiation induction. Small molecules that target signaling pathways, epigenetic modifications, and metabolic processes have been widely used

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to improve the transcription factor-based reprogramming or transdifferentiation [11–13]. To our surprise, a combination of small molecules can alone induce reprogramming and transdifferentiation without reintroduction of exogenous genes [13–17]. Small molecules are cell permeable and nonimmunogenic [14, 18, 19]. In addition, they are cost-effective, easily synthesized, preserved, and standardized [14, 18–20]. They hold distinct advantages over genetic manipulations: they are more convenient to use; their biological effects are rapid and often reversible and can be precisely controlled by changing the concentrations and combinations. Not only being useful to induce desired cell types in vitro but also can be small molecules used as drugs to promote endogenous regeneration in patients. Increasing small molecules are developed and identified to promote or directly induce reprogramming and transdifferentiation (Table 1). In this review, we focus on the effects of small molecules on reprogramming and transdifferentiation. The rapid and exciting progress in small molecules used in reprogramming and transdifferentiation field will undoubtedly speed up the step of biomedical studies and clinical translation.

Small molecules promote the transcription factor-based reprogramming by regulating signaling pathways

Pluripotent stem cells are characterized of high self-renewal and pluripotency [21–24]. Many signaling pathways are involved in maintaining the self-renewal and pluripotency. Thus, activation of these pathways by small molecules may contribute to pluripotency reprogramming (Figs. 1, 2). Wnt signaling pathway is involved in the control of self-renewal and pluripotency of pluripotent stem cells, because TCF3, a transcription repressor downstream of Wnt signaling, occupies the promoter regions of pluripotency-related genes and represses their expression [25, 26]. Activation of Wnt signaling can phosphorylate TCF3 and promote the expression of those pluripotency genes. Activating Wnt signaling directly by the GSK-3β inhibitor CHIR99021 has been shown to enhance iPSC reprogramming from mouse fibroblasts (increasing 0.2–0.4%) or neural stem cells by reprogramming factors [27, 28]. Moreover, CHIR99021 could replace Sox2 to induce iPSC reprogramming in the presence of only two reprogramming factors, Oct4 and Klf4 [27]. Likewise, another GSK-3β inhibitor Kenpaullone could increase reprogramming efficiency by approximately 10% and substitute for Klf4 during iPSC generation [29]. These results indicate that small molecules can function as exogenous genes to induce reprogramming. Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway is also essential for ESC pluripotency maintenance. Leukemia inhibitory factor (LIF) binds to its receptor LIF-R complexes containing the signaling transducer gp130, which then leads to phosphorylation and activation of associated JAK tyrosine kinases. Phosphorylated JAK activates the latent transcription factor STAT 3, whose activation maintains the ESC self-renewal [30]. Activating JAK-STAT was reported to promote the reprogramming of mouse neural stem cells or fibroblasts to iPSCs by three- to fourfold increase [31].

Pluripotent stem cells can differentiate under appropriate inductive signaling conditions. Accordingly, inhibition of signaling pathways associated with differentiation of pluripotent stem cells can maintain the pluripotency. Activation of the MAPK/ERK signaling pathway is known to facilitate ESC differentiation [32]. Blocking MAPK/ERK signaling with a potent MEK1/2 inhibitor PD0325901 was discovered to enhance the late stage of pluripotency reprogramming from mouse neural progenitor cells [33] (Fig. 2).

An early, strong induction of mesenchymal–epithelial transition (MET) is one of the critical events taking place during reprogramming, characterized by loss of mesenchymal characteristics and acquisition of epithelial features in mouse fibroblasts [34, 35]. Several reports have demonstrated that MET is a key early barrier to overcome during reprogramming [34, 35] (Fig. 2). Since TGF-β pathway induces epithelial-mesenchymal transition (EMT) [36], inhibition of TGF-β pathway can enhance the reprogramming. Indeed, inhibitors of the TGF-β pathway, such as SB431542, A83-01, and Repsox (or E-616452), have been used to enhance iPSC reprogramming or even replace reprogramming factors in various contexts [37, 38].

Small molecules promote the transcription factor-based reprogramming by regulating epigenetic barriers

Somatic cell reprogramming needs to overcome the epigenetic barriers that are established during development. Increasing evidence show that epigenetic barriers contribute to the low efficiency of iPSC generation [39]. Small molecules that target the epigenetic modifiers have been shown to improve reprogramming (Fig. 1). DNA methylation is generally associated with gene silencing [40–42]. In somatic cells, the regulatory regions of pluripotency genes are highly enriched with DNA methylation, and therefore, pluripotency genes are repressed. Inhibiting the DNA methyltransferases (DNMTs) that add DNA methylation with DNMT inhibitors 5-azacytidine (5-aza) and RG108 can activate the silenced pluripotency genes and

Table 1 Representative small molecules used in reprogramming and transdifferentiation

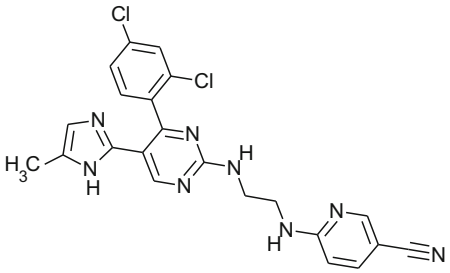
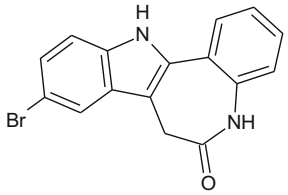
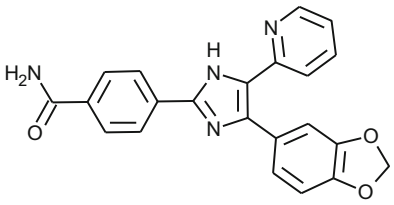
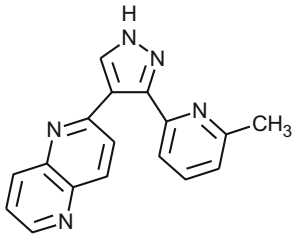
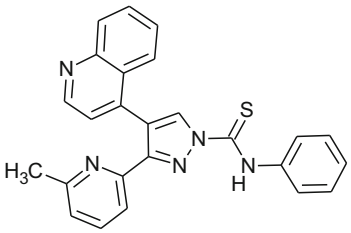
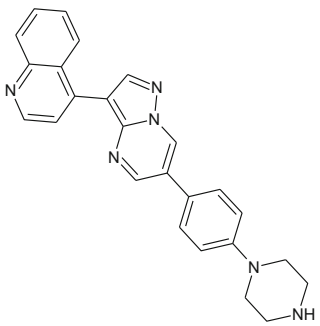
Name	Structure	Function
CHIR99021		GSK-3 inhibitor
Kenpaullone		GSK-3 and CDK1/cyclin B inhibitor
SB431542		TGF-β receptor ALK5/4/7 inhibitor
Repsox (E-616452)		TGFβ Receptor I kinase inhibitor
A83-01		TGF-β receptor ALK5/4/7 inhibitor
LDN193189		BMP receptor ALK2 and ALK3 antagonists

Table 1 contiuned

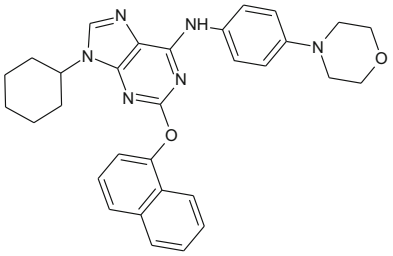
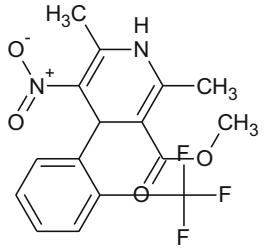
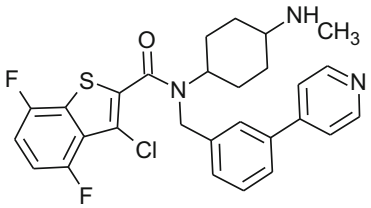
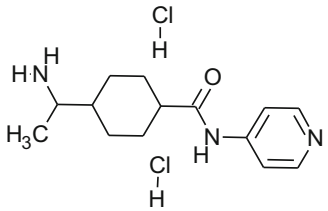
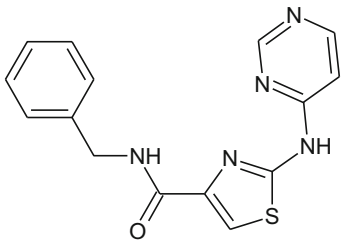
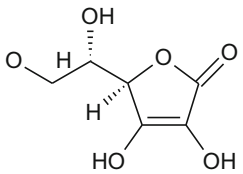
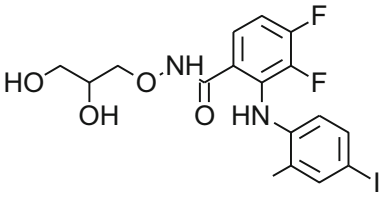
Purmorphamine		Hedgehog activator
SAG		Hedgehog activator
Hh-Ag 1.5		Hedgehog activator
Y-27632		ROCK inhibitor
Thiazovivin(Tzv)		Selective ROCK inhibitor
DAPT		γ-secretase inhibitor
PD0325901		Potent MEK1 and MEK2 inhibitor

Table 1 contiuned

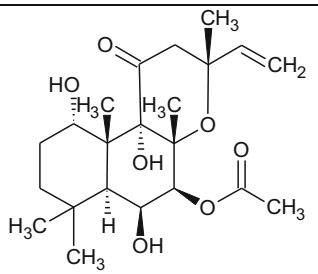
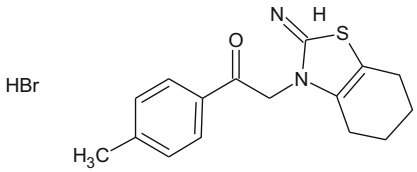
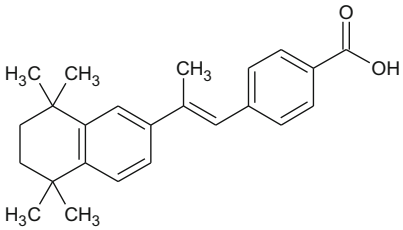
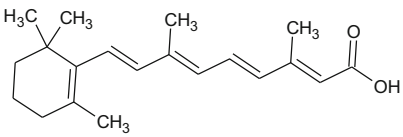
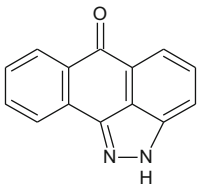
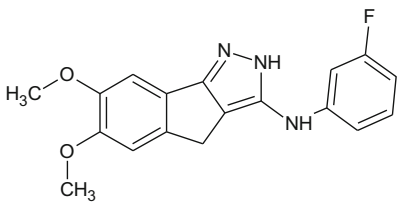
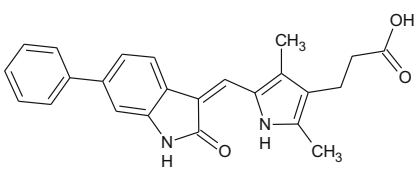
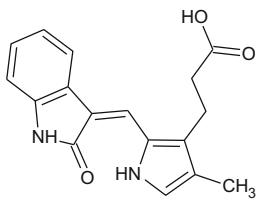
Forskolin		PKA activator
Pifithrin-α (PFT-α)		P53 inhibitor
TTNPB		Analog of retinoic acid
ATRA (RA)		Retinoic acid receptor agonist
SP600125		JNK inhibitor
JNJ-10198409		PDGF tyrosine kinase inhibitor
SU16F		PDGFR inhibitor
SU5402		FGFR, VEGFR, and PDGFR inhibitor

Table 1 contiuned

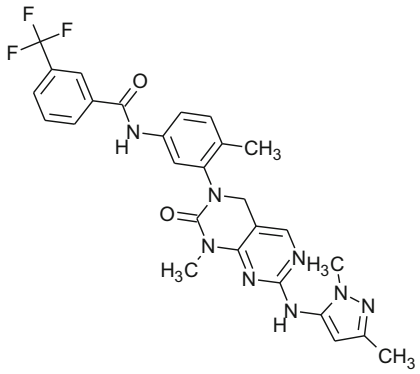
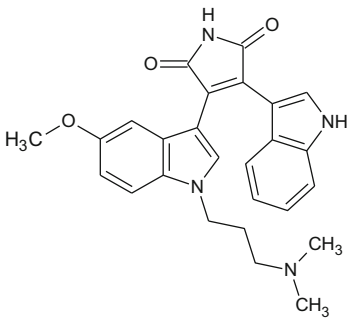
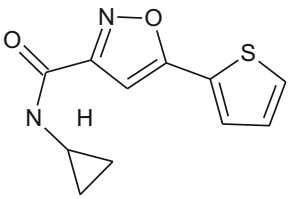
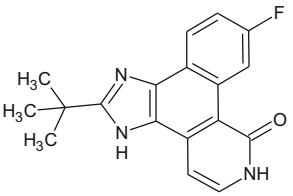
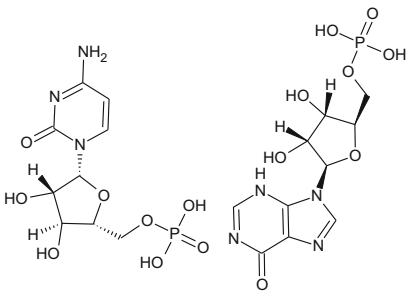
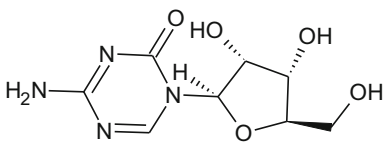
SC1		Dual inhibitor of extracellular signal-regulated kinase 1 and RasGAP
Go 6983		Broad spectrum protein kinase C inhibitor
ISX9		Neurogenic agent
JAK inhibitor 1 (JI1)		Janus kinase inhibitor
Poly(I:C)		Toll-like receptor 3 agonist
5-azacytidine		DNA methyltransferase inhibitor

Table 1 contiuned

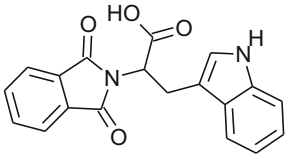
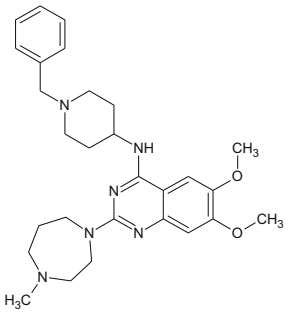
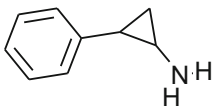
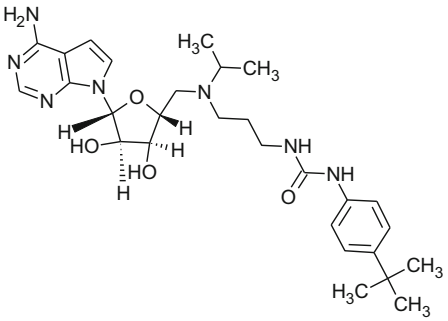
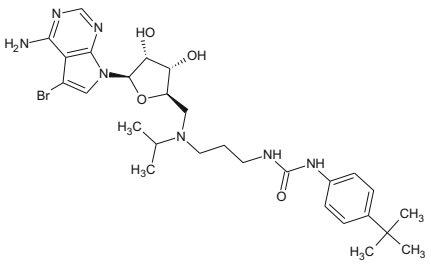
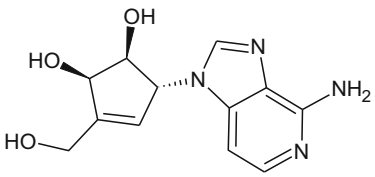
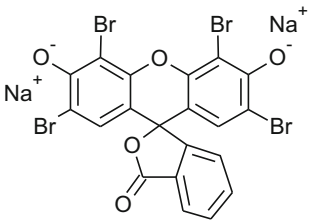
RG108		DNA methyltransferase inhibitor
BIX-01294		Histone lysine methyltransferase inhibitor
Tranylcypromine (Parnate)		Lysine specific demethylase 1 inhibitor
EPZ004777		Dot11 inhibitor
SGC0496		Dot11 inhibitor
DZNep		histone methyltransferase EZH2 inhibitor
AMI5		Protein arginine N-methyltransferase inhibitor

Table 1 contiuned

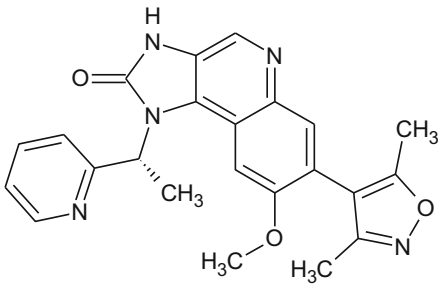
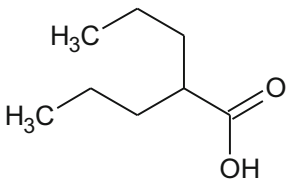
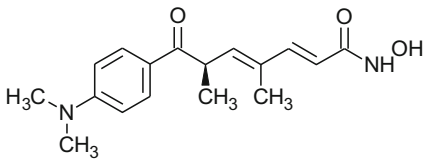
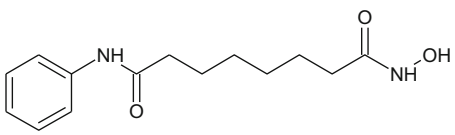
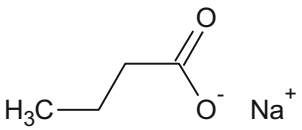
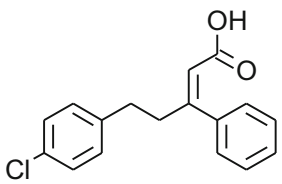
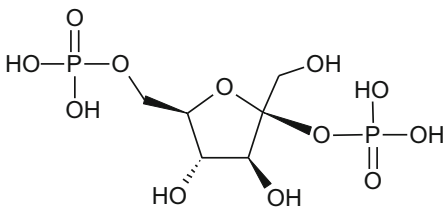
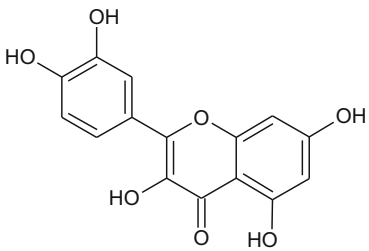
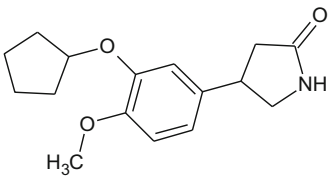
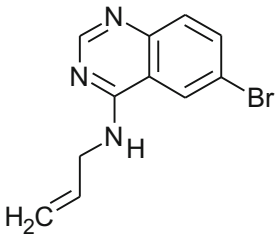
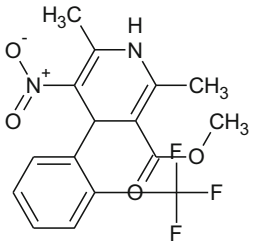
I-BET151		BET bromodomain inhibitor
VPA		HDAC inhibitor
TSA		HDAC inhibitor
SAHA		HDAC inhibitor
Sodium butyrate		HDAC inhibitor
PS48		PDK1 activator
Fructose 2,6-bisphosphate		Phosphofructokinase 1 activator
Quercetin		Hypoxia-inducible factor pathway activator

Table 1 contiuned

Rapamycin		mTOR inhibitor
SMER28		Autophagy modulator
(±) BayK 8644		L-type calcium channel agonist

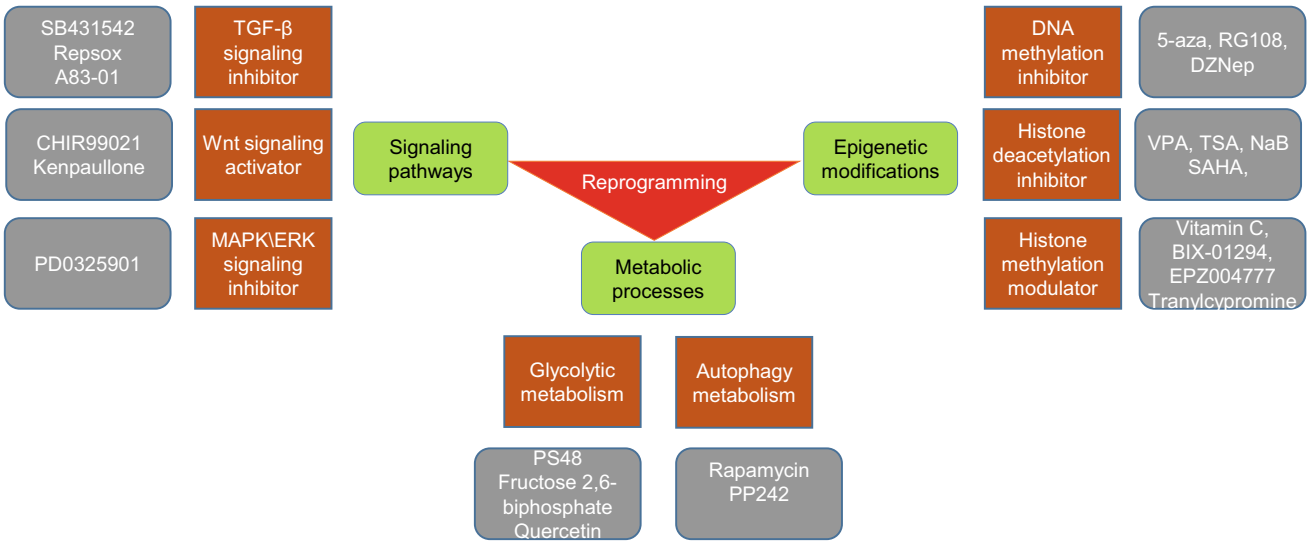


Fig. 1 Representative small molecules and their mechanisms for pluripotency reprogramming. Inhibition TGF-β signaling by its receptors SB431542, Repsox, and A83-01 facilitates the mesenchymal–epithelial transition and early stage reprogramming. Activation of Wnt signaling by a GSK-3β inhibitor (CHIR99021, Kenpaullone) can induce expression of pluripotency-associated genes. Inhibition of MAPK/ERK signaling by MEK inhibitor PD0325901 suppresses the differentiation of pluripotent stem cells and promotes the late-stage reprogramming. Reduction of DNA methylation levels in somatic cells by DNA methyltransferase inhibitors 5-azacytidine (5-aza), RG108, and 3-deazaneplanocin A (DZNep) enhances reprogramming. Increasing the histone acetylation level by histone deacetylase inhibitors valproic acid (VPA), trichostatin A (TSA), sodium butyrate (NaB), and suberanilohydroxamic acid (SAHA) enhances expression of pluripotency-associated genes and reprogramming efficiency. Vitamin C reduces repressive H3K9me2/3, H3K27me3 histone mark and increases active H3K4me3 at promoters of pluripotency-associated genes and reduces active H3K36me2/3 at promoters of cell senescence-associated genes, promoting reprogramming. BIX-01294, an inhibitor of H3K9me3 methyltransferase G9a, reduces repressive H3K9me3 mark and replaces Sox2 during reprogramming. EPZ004777 inhibits H3K79 histone methyltransferase Dot1l and improves the reprogramming rate. Tranylcypromine, an inhibitor of lysine-specific demethylase 1(LSD1), enables Oct4-based reprogramming. Promotion of glycolytic metabolism (PS48, fructose 2, 6-bisphosphate, quercetin) and autophagy metabolism (rapamycin, PP242, SMER28) can enhance reprogramming

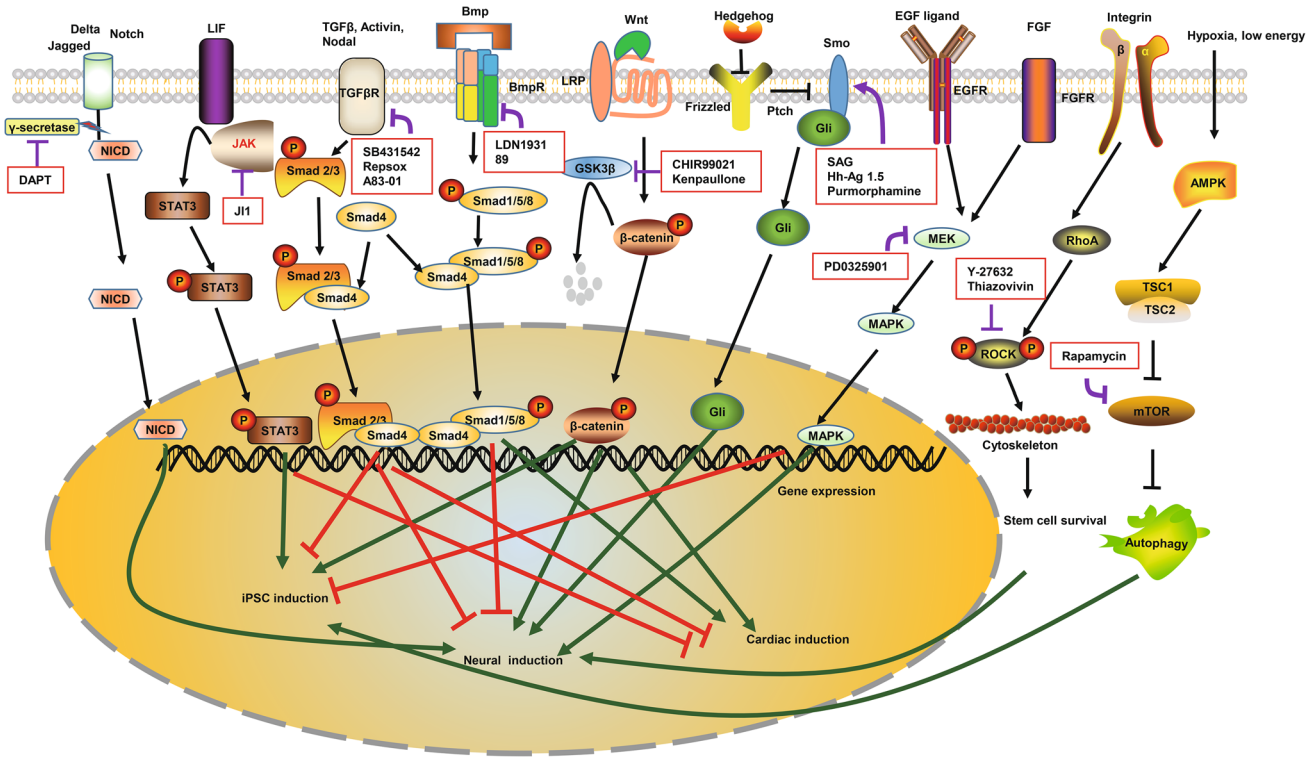


Fig. 2 Modulation of signaling pathways promotes the induction of iPSCs, cardiac cells, and neural cells from somatic cells. The Notch, JAK-STAT, TGF-β, Bmp, Wnt, Hedgehog, MAPK/ERK, ROCK, and mTOR signaling pathways and the small molecules (*red rectangle*) that target them are simply presented in the diagram. The inhibition and activation of these signaling pathways regulate gene expressions and control the cell fate determination of somatic cells. *Green arrows* indicate the contribution to cell induction and *red blunt-headed arrows* indicate the inhibition to cell induction. *Blue arrows* indicate the activation to signaling pathways and *blue blunt-headed arrows* indicate the inhibition to signaling pathways

promote reprogramming [43, 44]. 5-aza inhibits DNMTs at low doses but causes toxicity at high doses by incorporating into DNA and RNA strands [45]. It, therefore, needs to determine the optimal concentration during using 5-aza to enhance reprogramming. In contrast, RG108 is less damaging to DNA because it binds directly to the DNMT active site and is not a nucleoside DNMT inhibitor [46]. An alternative strategy for reducing DNA methylation is to deplete the substrate of DNMTs. DNMTs have common S-adenosylmethionine (SAM) binding sites where SAM provides methyl groups. Consequently, inhibiting the synthesis of the DNMT substrate SAM with small molecule 3-deazaneplanocin A (DZNep) reduces DNA methylation and promotes the late stage of reprogramming by 65-fold [14].

Histones can be post-translationally modified, such as methylation and acetylation. Histone methylation contributes to gene activation or repression, whereas histone acetylation is generally associated with gene activation [47–50]. Modulation of histone methylation and acetylation presents an effective approach for improving reprogramming. In the genome of mouse embryonic fibroblasts, the widespread repressive H3K9me2/3 (dimethylation and trimethylation of histone 3 lysine 9) marks block the OSKM-based reprogramming [51]. The supplementation of small molecule vitamin C potentially enhanced the OSKM-based reprogramming of murine and human fibroblasts, at least in part because its treatment reduced the H3K9me2/3 marks through increasing the expression of several H3K9 demethylases that can demethylate H3K9me2/3 [51–53]. In addition, CYT296, when added to the reprogramming medium, reduced H3K9me3 and increased the OSKM-based reprogramming for >10-fold [54]. BIX-01294, a specific inhibitor of H3K9me3 methyltransferase G9a, could reprogram mouse embryonic fibroblasts to iPSCs with only transduction of Oct4 and Klf4, suggesting BIX-01294 may replace Sox2 [44]. H3K27me3 (trimethylation of histone 3 lysine 27) mark inhibits the reprogramming as well. Vitamin C treatment decreased H3K27me3 at promoter regions of pluripotency genes such as Zfp42 (ZFP42 zinc finger protein), Ddx4 (DEAD-box helicase 4), and Nanog, and consequently facilitated their expression during the transition of pre-iPSC to iPSC [55]. H3K79 methylation inhibits reprogramming, and reducing H3K79 methylation with EPZ004777, an inhibitor of H3K79 histone

methyltransferase Dot1l, improved the reprogramming rate for about fourfold [56].

In addition to inhibition of histone methyltransferases, inhibition of histone demethylases can promote reprogramming. H3K4 methylation is often related to gene activation. Tranylcypromine (or Parnate) is an inhibitor of lysine-specific demethylase 1 (LSD1) that demethylate H3K4 methylation, and its treatment promoted OSK-or Oct4-mediated iPSC generation from mouse fibroblasts (by approximately 20-fold) [57] or OK-mediated iPSC generation from human keratinocytes [27]. Mechanically, Tranylcypromine-mediated inhibition of LSD1 promoted the expression of exogenous OSK genes and metabolic switch [28]. Vitamin C can maintain H3K4me2 and acquire H3K4me3 at the imprinted Dlk-Dio3 locus that is responsible for achieving full pluripotency. As a result, vitamin C treatment facilitated Dlk-Dio3 transcription and fully reprogrammed mouse B-lymphocytes to iPSCs that were able to generate all iPSC-derived mice [58]. Although H3K36me2/3 marks are usually transcriptional active, decreasing H3K36me2/3 by vitamin C-mediated upregulation of H3K36 demethylases Jhdm1a/1b led to an increased generation of mouse iPSCs (from 1% to about 4%). This increased reprogramming is at least in part due to reduced active H3K36me2/3 marks from the Ink4/Arf locus that is required for cell senescence, thereby repressing the Ink4/Arf-mediated cell senescence during reprogramming [59]. This study indicates that removing active histone marks from genes inhibiting reprogramming provides an alternative strategy to promote reprogramming.

Histone acetylation generally correlates with gene activation. Increasing the level of histone acetylation by HDAC inhibitors such as valproic acid (VPA) [44, 60], sodium butyrate (NaB) [61], trichostatin A (TSA) [60, 61] and suberoylanilide hydroxamic acid (SAHA) [62] has been demonstrated to promote pluripotency reprogramming.

Small molecules promote the transcription factor-based reprogramming by modulating metabolism

Many stem cells and highly proliferative cells rely more heavily on aerobic glycolysis to support their proliferation, as compared to somatic cells. For instance, ESC self-renewal is associated with reduced oxidative phosphorylation and increased glycolysis [63, 64]. In support of this, hypoxic conditions enhanced reprogramming rate [65]. Strengthening the transition from oxidative phosphorylation to glycolysis by small molecules is potent to promote pluripotency reprogramming (Fig. 1). PS48, an activator of 3′ phosphoinositide-dependent kinase 1 that can advance metabolic conversion to glycolysis, increased the rate of Oct-4 mediated reprogramming by about 15-fold [66]. In agreement, many small molecules that promote glycolysis more directly, such as fructose 2, 6-bisphosphate (an activator of phosphofructokinase 1) and quercetin (increasing HIF-1 activity), raised the reprogramming efficiency [66]. Conversely, a glycolysis inhibitor 2-deoxy-D-glucose suppressed the reprogramming process [67].

In the course of reprogramming, the cellular contents change a lot, with a dramatic turnover of cytoplasmic macromolecules and organelles. The autophagic metabolism is found to regulate reprogramming by degrading those proteins and organelles. Small molecules activating autophagy have been identified to enhance reprogramming by the fivefold increase, such as rapamycin and PP242 [68]. A transient promotion of Atg5-dependent autophagy by Sox2-dependent early downregulation of rapamycin (mTOR) is required for iPSC reprogramming [69]. Atg5-independent autophagy mediating mitochondrial clearance that is critical for the transition of oxidative phosphorylation to glycolysis, likewise, plays a crucial role in achieving pluripotency. Activating Atg5-independent autophagy with small molecules can promote reprogramming [70]. Moreover, Atg3-dependent autophagy contributes to acquisition and maintenance of pluripotency during reprogramming [71].

Only small molecules reprogram somatic cells to pluripotency

Pure small molecules have the potential to induce iPSC reprogramming alone (Table 2). A combination of small molecule cocktail containing VPA, CHIR99021, Repsox, tranylcypromine, Forskolin, and DZNep could induce mouse fibroblasts to iPSCs at a frequency up to 0.2% [14], avoiding the transgene delivery. The chemical treatment did not directly activate the endogenous expression of Oct4, Sox2, Klf4, and c-Myc. Instead, a high expression of extraembryonic endoderm (XEN) cell genes was observed after chemical induction [14]. By tracing the reprogramming process, the group found that almost all the iPSC arose from a type of induced epithelial cell, called XEN-like cells, which express a high level of XEN genes Sall4, Gata4, Gata6, Sox17, and Sox7, suggesting that chemical reprogramming involves an XEN-like intermediate state during iPSC generation. Mouse neural stem cells and intestinal cells were likewise induced to pluripotency by a similar combination of small molecules [18]. Similar to fibroblasts, neural stem cells and intestinal cells underwent an XEN-like stage before becoming iPSCs [18]. Moreover, chemical screening showed that additional retinoic acid receptor α agonist AM580 and Dot1l inhibitor EPZ00477 could promote the transition from mouse fibroblasts to

Table 2 Small molecules alone induce pluripotency reprogramming and transdifferentiation

Cell types		Small molecules	References
Starting cell	Ending cells		
Mouse fibroblasts	iPSC	VPA, CHIR99021, Repsox, Forskolin, Tranylcypromine, DZNep	[14, 39]
Mouse fibroblasts	iPSC	VPA, CHIR99021, Repsox, Forskolin, Tranylcypromine, DZNep, AM580, EPZ004777, 5-aza-dC, SGC0946	[39]
Mouse endothelial cells	iPSC	VPA, CHIR99021, Repsox, Tranylcypromine, Forskolin, AM 580, DZNep	[18]
Mouse neural stem cells	iPSC	VPA, CHIR99021, Repsox, Tranylcypromine, Forskolin, EPZ004777, DZNep	[18]
Human urine cells	iPSC	Pifithrin-α, A83-01, CHIR99021, Thiazovivin, NaB, PD0325901	[40]
Mouse fibroblasts	Cardiomyocytes	VPA, CHIR99021, Repsox, Forskolin, Tranylcypromine, TTNPB	[16]
Mouse fibroblasts	Cardiomyocytes	CHIR99021, A83-01, Forskolin, SC1, (±) BayK 8644	[41]
Human fibroblasts	Cardiomyocytes	CHIR99021, A83-01, BIX-01294, AS8351, SC1, Y-27632, OAC2, SU16F, JNJ-10198409	[19]
Mouse fibroblasts	Neurons	CHIR99021, Forskolin, ISX9, I-BET151	[42]
Human fibroblasts	Neurons	VPA, CHIR99021, Repsox, Forskolin, SP600125, GO6983, Y-27632	[17]
Human fibroblasts	Neurons	CHIR99021, SB431542, LDN193189, PD0325901, Pifithrin-α	[43]
Mouse fibroblasts	Neurons	SB431542, RA	[44]
Mouse fibroblasts	Dopaminergic neurons	SB431542, Noggin, RA	[45]
Mouse astrocytes	Neurons	Repsox, VPA	[15]
Human astrocytes	Neurons	VPA, CHIR99021, Repsox, Forskolin, I-BET151, ISX9	[46]
Mouse fibroblasts	Neural progenitor cells	VPA, CHIR99021, Repsox	[47]
Human urinal cells	Neural progenitor cells	VPA, CHIR99021, Repsox	[47]
Mouse fibroblasts	Neural stem cell	VPA, CHIR99021, A83-01, BIX-01294, RG108, Vitamin C or BIX-01294, RG108, PD0325901	[48]
Mouse fibroblasts	Neural stem cell	CHIR99021, A83-01, RA, LDN193189, Hh-Ag 1.5, Tranylcypromine, SMER28, RG108	[49]
Mouse fibroblasts	Neural stem cells	A83-01, Thiazovivin, Purmorphamine, VPA	[50]
Mouse fibroblasts	Myocytic, glial, adipocytic cells	Repsox, Tranylcypromine, CHIR99021, Forskolin	[51]
Mouse fibroblasts	Astrocytes	VPA, CHIR99021, Repsox, Tranylcypromine, OAC1	[52]
Human fibroblasts	Endothelial cells	Poly I:C	[53]
Pig fibroblasts	Insulin-secreting cells	5-azacytidine	[54]
Human fibroblasts	Insulin-secreting cells	5-azacytidine	[55]
Human fibroblasts	Insulin-secreting cells	Nicotinamide	[56]
Human fibroblasts	Mesenchymal stem cells	SP600125, SB202190, Go 6983, Y-27632, PD0325901, CHIR99021	[57]
Rat and mouse hepatocytes	Bipotent liver progenitor cells	Y-27632, A83-01, CHIR99021	[58]

XEN-like cells, and that Dot1l inhibitor SGC0946 and DNMT inhibitor 5-aza-dC enhanced the progression of XEN-like cells to iPSCs [72]. Regarding changes in gene expression, the chemical reprogramming process differs from transcription factor-based reprogramming. Transcription factor-based reprogramming process involves gradual upregulation of pluripotency genes and transient expression of genes associated with the pre-implantation embryo, such as DPPA3 and DNMT3L, without expression of XEN-like cell genes [43, 73–75], indicating a reversal of early embryonic development. Given that XEN-like cells are one of the three specialized cell types in the early embryo, chemical reprogramming may also undergo a reversal of the embryonic development, though differing from the transcription factor-based reprogramming.

Small molecule-mediated transdifferentiation

Transdifferentiation of one cell type to another has been widely achieved by ectopic expression of lineage-specific transcription factors (Fig. 3). In recent years, transdifferentiation has been induced by alternative strategies. One strategy employs transient overexpression of pluripotency

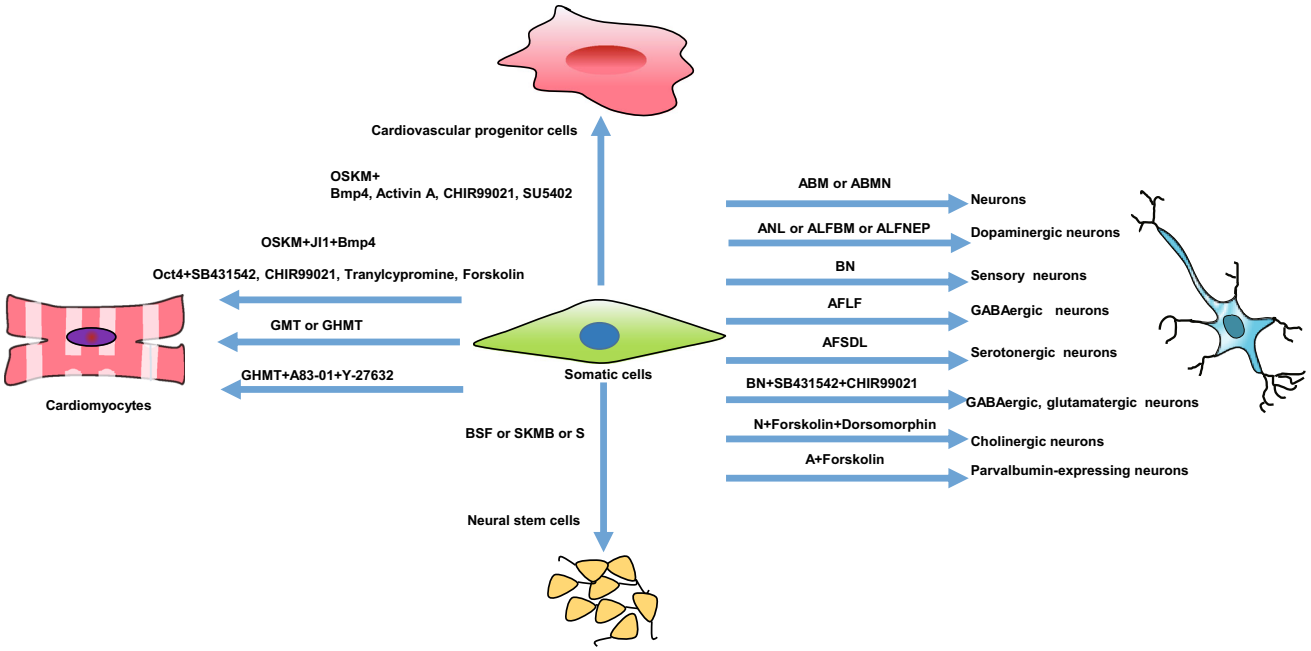


Fig. 3 Small molecules promote transcription factor-based transdifferentiation. Fibroblasts can be converted to cardiomyocytes or cardiovascular progenitor cells by transient expression of OSKM (Oct4, Sox2, Klf4, c-Myc) or only Oct4 combined with treatment with small molecules. Alternatively, ectopic expression of cardiac-associated transcription factors GMT (Gata4, Mef2c, Tbx5) or GHMT (Gata4, Hand2, Mef2c, Tbx5) can induce cardiomyocytes. Small molecules (A83-01, an inhibitor of TGF-β receptor inhibitor, and Y-27632, a ROCK inhibitor) can promote GHMT-based cardiac conversion. Fibroblasts can be induced to neurons (ABM, Ascl1, Brn2, Myt1l; ABMN; Ascl1, Brn2, Myt1l, NeuroD1), specific neuronal subtypes (ANL, Ascl1, Nurr1, Lmx1a; AFLBM, Ascl1, Lmx1a, Foxa2, Brn2, and Myt1l; ALFNEP, Ascl1, Lmx1a, Foxa2, Nurr1, En1, and Pitx3; BN, Brn3a, Nrg1 or Ngn2; AFLF, Ascl1, Foxa2, Lmx1b, and FEV; AFSDL, Ascl1, Foxg1, Sox2, Dlx5, Lhx6), neural stem cells (BSF, Brn2, Sox2, FoxG1; SKMB, Sox2, Klf4, c-Myc, Brn4; S, Sox2) by ectopic expression of neural-associated transcription factors. Small molecules combined with transcription factors (BN; Ascl1, Ngn2; N, Ngn2; A, Ascl1) can generate specific neuronal subtypes

transcription factors coupled with small molecules. Another strategy only uses a combination of small molecules, not requiring the overexpression of pluripotency transcription factors or lineage-specific transcription factors (Table 2).

Small molecules together with pluripotency transcription factors induce cardiac cells

Long-term transfection of pluripotency transcription factors generates pluripotent stem cells. However, the progression to pluripotency can be blocked by terminating the expression of exogenous genes. The mechanical analysis illustrates that early, transient expression of pluripotency transcription factors creates an epigenetically unstable state, where the cell fate can be directed by exogenous inductive signals. In agreement with this idea, cardiomyocytes are obtained from fibroblasts by transient expression of pluripotency transcription factors combined with modulation of signaling pathways and epigenetics (Fig. 3).

Mouse embryonic fibroblasts were directly converted to cardiomyocytes after transient expression of OSKM and sequential treatment with small molecule JI1 (a JAK-STAT inhibitor) and Bmp4 over 12 days, at a frequency of 1% [76]. Specifically, JI1 and Bmp4 promoting cardiac commitment redirected the fate of partially reprogrammed cells to cardiac cells. Activation of JAK-STAT signaling is required for pluripotency maintenance and differentiation of cardiac precursors [77]. In this case, JI1 may prevent the reprogrammed cells from entering into pluripotency or facilitate the expansion of cardiac precursors, which is consistent with a transient cardiac precursor intermediate observed in the conversion process. The subsequent Bmp4 treatment may advance the cardiac differentiation of the cardiac precursor intermediate because Bmp4 treatment can drive cardiac induction from nascent precursors, ESCs, or iPSCs [78, 79].

The transition of cardiac precursor intermediate provides a possibility of generating cardiac progenitors by inhibiting differentiation. As expected, another study obtained cardiovascular progenitor cells (CPC) from mouse fibroblasts (about 70%) by transient expression of pluripotency transcription factors and subsequent treatment with small molecules (CHIR99021 and SU5402) and proteins (Bmp4 and Activin) [4]. Bmp, TGF-β, and Wnt

signaling can synergistically induce mesoderm and subsequent CPC formation from pluripotent stem cells [79, 80]. Particularly, Wnt signaling stimulates mesoderm induction from early stem cells and inhibits cardiac differentiation of the late-stage CPC [81, 82]. In this study, activation of Bmp signaling (Bmp4), TGF-β signaling (Activin), and Wnt signaling (CHIR99021) therefore, may play major roles in the induction of CPC. To maintain the CPC, additional molecule SU5402 is added to prevent further differentiation of the induced CPC, because SU5402 can inhibit FGF, VEGF, and PDGF signaling pathways that are required for differentiation of CPC [79, 83].

Substantial efforts have been made to reduce the use of pluripotency transcription factors by small molecules. In the presence of only Oct4, small molecules, including SB431542, CHIR99021, Forskolin, and Tranylcypromine, could reprogram mouse fibroblasts into cardiomyocytes (with about 100 contracting clusters from 10,000 fibroblasts) [84]. Inhibiting TGF-β signaling pathway by SB431542 can promote MET during reprogramming and replace Sox2 [34, 85]. Moreover, TGF-β signaling inhibits cardiac specification and differentiation from the ESC-derived mesoderm cells [86, 87]. Therefore, SB431542-mediated inhibition of TGF-β signaling may contribute to cardiac induction in this case. Wnt signaling activation by CHIR99021 initiates reprogramming during iPSC generation [27]. Furthermore, Wnt signaling activation promotes induction and proliferation of cardiovascular progenitors [93–96]. The increased cAMP by Forskolin can lead to PKA-mediated phosphorylation of connexin and promote electrical cell-to-cell coupling in cardiac cells, increase the beating frequency of cardiomyocytes [88, 89]. Additionally, differentiation of mouse ESCs into cardiomyocytes is stimulated by cAMP treatment [90]. These data collectively demonstrate that Forskolin-mediated increase in cAMP levels facilitates the conversion of fibroblasts to cardiomyocytes. Tranylcypromine may increase the level of H3K4 methylation that is associated with gene activation, thereby strengthening the initial epigenetic changes in human fibroblasts [27].

Small molecules promote cardiac transdifferentiation by lineage transcription factors

Ectopic expression of three cardiac-enriched transcription factors (Gata4, Mef2c, and Tbx5, GMT) or four transcription factors (Gata4, Hand2, Mef2c, and Tbx5, GHMT) can derive cardiac cells from mouse fibroblasts [3, 91] (Fig. 3). However, so far, these strategies are mostly inefficient. Inhibiting the profibrotic TGF-β (using A83-01) or Rho-associated kinase (ROCK) (using Y-27632) pathways could enhance the GMT-or GHMT-mediated conversion of mouse fibroblasts to cardiomyocytes by eight- or sevenfold, respectively [92]. In addition, inhibition of Notch signaling by DAPT could enhance the conversion (>70%) of mouse fibroblasts into cardiomyocytes by GHMT [93]. A combination of SB431542 and XAV939 (a Wnt inhibitor) increased cardiomyocyte generation efficiency eightfold from mouse fibroblasts transfected with GMT [94]. By inhibiting H3K4 methyltransferase Mll1, small molecule MM408 could improve the transdifferentiation of mouse embryonic fibroblasts and cardiac fibroblasts into cardiomyocytes with a onefold increase [95].

Only small molecules induce somatic cells to cardiac cells

Without genetic manipulation, only small molecules can induce somatic cells into cardiac cells (Table 2). A chemical cocktail (CHIR99021, Repsox, Forskolin, VPA, Tranylcypromine, TTNPB, CRFVPT) led to cardiomyocyte induction from mouse fibroblasts, with 100 contracting clusters obtained from 50,000 fibroblasts [16]. Among these compounds, CHIR99021 (activating Wnt signaling), Repsox (inhibiting TGF-β signaling), and Forskolin (increasing cAMP) are the core chemicals for cardiac induction. Moreover, the supplementation of phosphodiesterase (PDE) 4 inhibitors (Rolipram and Cilomilast) could amplify the cardiomyocyte induction with CRFVPT. Rolipram had the best effect for enhancing the cardiomyocyte induction, and Cilomilast was also highly effective [16]. The beneficial effects of PDE 4 inhibitors may depend on increased intracellular cAMP levels [16]. Another research group used another chemical cocktail (CHIR99021, A83-01, Forskolin, SC1, and (±) Bayk 86244) to induce mouse fibroblasts (27%) to cardiomyocytes [96]. A83-01 has similar effects with Repsox. The latter study supplemented two different small molecules SC1 and (±) Bayk 86244. SC1 inhibits ras GTPase activating protein (Ras-GAP)/extracellular signal-regulated kinase (ERK), while SC1 effects on cardiomyocyte induction cannot be replaced by PD0325901 (an MEK/ERK specific inhibitor) or arachidonic acid (a non-specific Ras-GAP inhibitor). Thus, future study should further elucidate the roles of SC1. (±) BayK 8644, a Ca^{2+} channel agonist, stimulates beating frequency [97]. The two molecules might contribute to cardiomyocyte maturation. Gao and his colleagues reported a cardiac induction from human fibroblasts using nine molecules, including CHIR99021, A83-01, BIX-01294, AS8351, SC1, Y-27632, OAC2, SU16F, and JNJ-10198409 [19]. Approximate 6% of the small molecule-treated cells become

cardiomyocytes. Among them, CHIR99021, A83-01, BIX-01294, AS8351, SC1, Y-27632, and OAC2 were sufficient to induce cardiomyocytes. The epigenetic modulator OAC2 can activate Oct4 and facilitate the chemical cardiomyocyte conversion. AS8351 regulates the epigenetic modifications through inhibiting the JmjC-domain-containing histone demethylase (JmjC-KDM) family member KDM5, whose inhibition sustains the active histone mark (i.e., H3K4 methylation) in the genomic sites. SU16F and JNJ-10198409, two inhibitors of the PDGF pathway, could speed-up the loss of fibroblast genes and increase the yield of cardiomyocytes.

Small molecules improve the neural conversion efficiency induced by transcription factors

Although ectopic expression of transcription factors can induce neural conversion (Fig. 3), this strategy is challenged by safety issues and inefficiency. Accumulating evidence shows that modulating signaling pathways associated with the neural development and differentiation by small molecules can increase the conversion efficiency or even replaces some transcription factors (Fig. 3).

Pfisterer et al. identified six small molecules that could increase the neural conversion (>6-fold) of human fibroblasts induced by transcription factors Ascl1, Brn2, and Myt1L [98]. The six small molecules contain Kenpaullone (GSK 3β inhibitor), PGE2 (cAMP/PKA modulator), Forskolin, BML210 (HDAC inhibitor), Aminoresveratrol sulfate (SIRT1 activator), and PP2 (Src kinase inhibitor). Another study demonstrated that inhibiting TGF-β signaling by its inhibitor (SB431542) and stimulating Wnt signaling by a GSK-3β inhibitor (CHIR99021) significantly enhanced the conversion of human fibroblasts into neurons after transduction with two neural transcription factors Ascl1 and Ngn2, with yields up to >200% [99]. In the study, the induced neurons were a heterogeneous population of GABAergic (20%) neurons, glutamatergic (35%) neurons, and a low percentage of other neuronal subtypes, without cells expressing motor neuron-related markers ChAT (choline acetyltransferase) or HB9. In contrast, another study used two small molecules (Forskolin and Dorsomorphin) combined with a single neural transcription factor Ngn2 to convert human fetal lung fibroblasts into cholinergic neurons highly expressing ChAT and HB9 (up to 99% of Ngn2-expressing cells) [100]. Only Ngn2 overexpression itself did not cause significant changes in morphology or proliferation of human fetal fibroblast; the addition with Forskolin and Dorsomorphin rapidly altered the morphology. These authors further revealed that Ngn2 acted as a pioneer factor and that Forskolin and Dorsomorphin changed the epigenetic state by increasing chromatin accessibility and H3K27 acetylation [101]. Moreover, Forskolin treatment and Ascl1 transduction induced 80% of mouse fibroblasts to parvalbumin-expressing neurons [102]. Why do the three research groups obtain different neuronal subtypes with similar transcription factors? Ascl1 and Ngn2 have opposing roles in cell fate determination in the different brain and spinal cord regions [103, 104]. The final expression levels of the two transcription factors within a heterogeneous cell population may result in a mixture of neuronal subtypes. Accordingly, the use of a single transcription factor will determine a more homogenous neuronal subtype. The different small molecules may contribute to specific neuron induction induced by transcription factors. For example, when CHIR99021 was added in the Ngn2-based transdifferentiation, no HB9 was detected [100]. This may be due to that GSK 3β is critical for phosphorylation of Ngn2 and motor neuron specification. These data suggest that neuronal identity is determined and maintained by a coordinated action of intrinsic transcription factors and extrinsic signaling pathways. Based on the transcription factor-based neural conversion, more small molecules are likely screened to reduce the use of exogenous transcription factors, improve the efficiency, or help to generate specific neuronal subtypes.

Small molecules-based conversion of somatic cells to neural cells

Small molecules can improve the efficiency or reduce the use of exogenous transcription factors during neural conversion. However, considering the clinical safety issues, it will be desirable to induce direct conversion by small molecule without the exogenous gene introduction. Based on the knowledge of the neural development- and differentiation-associated signaling pathways and the mechanisms of the pluripotency reprogramming process, various combinations of small molecules have been identified to induce neural cells (Table 2).

Conversion into neural stem/progenitor cells

Cheng and his colleagues found a series of chemical cocktails containing VPA, CHIR99021, and Repsox could induce conversion of somatic cells (including mouse embryonic fibroblasts, mouse tail-tip fibroblasts, human urinary cells) to neural progenitor cells under a physiological hypoxic condition (5% O_2), with about 30 neural colonies generated from 200,000 cells [105]. Zheng et al. reported conversion of mouse embryonic fibroblasts into neural stem cells by four chemicals, including VPA, A83-01, Thiazovivin, and Purmorphamine [106]. Comparing the

two combinations, the effects of activating Wnt signaling by GSK-3β inhibitor (CHIR99021) on neural stem cell induction might be replaced by Rho-associated kinase (ROCK) inhibitor (Thiazovivin) and SHH pathway activation (Purmorphamine), which is in accordance with the promoting effects of Wnt signaling and SHH signaling on neural progenitor cell proliferation [107, 108]. In addition, ROCK inhibition enhances the viability of neural cells by protecting them from apoptosis [109]. TGF-β inhibition enhances MET, and epigenetic modulation by VPA promotes reprogramming efficiency. By contrast, another group employed nine molecules combining bFGF to convert mouse embryonic fibroblasts into neural stem cells at a conversion efficiency of about 25% [110]. These chemicals include LDN193189 (an inhibitor of Bmp type I receptor ALK2/3), A83-01, CHIR99021, Hh-Ag 1.5 (a potent Smo agonist), retinoic acid, RG108, tranylcypromine, SMER28 (an autophagy modulator) [110]. bFGF and Hg-Ag 1.5 played important roles in the induction because omitting bFGF or Hh-Ag 1.5 reduced the NSC induction significantly. The function of bFGF depends on the major downstream MAPK pathways and the phosphatidylinositol 3-kinase (PI3K) pathway, as blocking MAPK or PI3K impaired the efficiency under the nine small-molecule induction condition. Inhibiting SHH pathway by a Smo antagonist also reduced the efficiency, suggesting the importance of activating SHH pathway in neural induction. Mechanical analysis revealed that the treatment of nine small molecules activated the bFGF downstream transcription factor Elk1 and protected SHH pathway downstream transcription factor Gli2 from degradation, resulting in the binding of Elk1 and Gli2 to the promoter of Sox2 and activating its transcription. Accordingly, bFGF and Hh-Ag 1.5 may regulate the Sox2 expression through activating MAPK and SHH pathways. Another combination of small molecules (VPA, BIX-01294, RG108, PD0325901, CHIR99021, vitamin C, A83-01) was found to induce up to 2% of mouse embryonic fibroblasts into neural stem cells [111]. By withdrawal of individual molecules from the combination, the author identified that three molecules (BIX-01294, RG108, and PD0325901) were important for the induction to occur, though the underlying mechanisms are still unknown. In all, all these combinations support the model that activating the signaling pathways associated with neural induction and promotion of proliferation, inhibiting neural differentiation, and modulating epigenetic modifications together can induce neural stem cells from somatic cells.

Conversion into neuronal cells

Considering that in vitro the differentiation of neural stem cells into neurons is still time-consuming and in vivo differentiation into neurons is difficult, some researchers try to establish a direct conversion into neuronal cells by small molecules. Wu and his colleagues converted about 20% normal and Alzheimer's disease human fibroblasts into neuronal cells by chemical cocktails (VPA, CHIR99021, Repsox, Forskolin, SP600125, GO6983, Y-27632) [17]. Among them, VPA, CHIR99021, Repsox, and Forskolin (VCRF) could induce a bipolar neuron-like cell morphology, and some of these bipolar neuron-like cells expressed the neuronal marker TUJ1. However, the round and prominent cell bodies are unlike a typical neuron morphology, indicating a partial conversion. SP600125 (JNK inhibitor), GO6983 (PKC inhibitor), and Y-27632 (ROCK inhibitor) speeded neuronal maturation. Most of these induced neurons are glutamatergic neurons in this induction system. Li et al. showed that using four molecules (CHIR99021, Forskolin, ISX9, I-BET151) directly converted mouse fibroblasts into neuronal cells, with a yield of up to >90% being TUJ1-positive [112]. ISX9 activated master neural genes (Ngn2, NeuroD1, NF-H, Tau, and Syn2), and I-BET151 suppressed the endogenous fibroblast-fate program. This study provides a direct conversion strategy by activating desirable cell-type-specific master gene expression and silencing initial cell-type-specific master gene expression. Dai et al. applied SB431542, CHIR99021, PD0325901 (an inhibitor of MEK-ERK), Pifithrin-α (a p53 inhibitor), LDN-193189, and Forskolin to induce neurons from human fibroblasts (induction efficiency >80%) after 21 days [113]. The Pifithrin-α plays a critical role in this combination because removal of Pifithrin-α from the cocktail resulted in no generation of neuronal cells. Moreover, small molecules are capable of generating neural subtypes from somatic cells. Recently, mouse embryonic fibroblasts have been converted into dopaminergic neurons by small molecules and specific growth factors, with about 18% $TH^+/TUJ1^+$ cells [114]. This protocol involves initial ten days of treatment with SB431542, Noggin, bFGF, EGF, GDNF, and RA and later two–four weeks of treatment with bFGF, EGF, RA, SHH, and FGF8.

In addition to fibroblasts, other somatic cells can be converted to neurons by chemical cocktails. Cheng et al. used VPA, CHIR99021, and Repsox that were reported to induce neural progenitor cells, to drive mouse astrocytes into neurons (>20%) [15]. In this case, the VPA concentration was changed, and the basal medium was supplemented with BDNF and GDNF. VPA was necessary for generating neuroblasts from astrocytes and significantly activated the expression of NeuroG2 and NeuroD1. Zhang et al. designed a chemical induction approach to convert human astrocytes (about 60%) into neurons within 8–10 days [115]. They screened nine small molecules that inhibit astrocyte-signaling pathways but activate neuronal

signaling pathways, when added in a stepwise manner, resulting in astrocyte–neuron conversion. Human astrocytes were first treated with LDN193189, SB431542, TTNPB, and Thiazovivin for 2 days. The LDN193189, SB431542, and TTNPB aimed to inhibit glial signaling pathways and activate neuronal signaling pathways. Afterwards, LDN193189, SB431542, and TTNPB were replaced with CHIR99021, DAPT, and VPA. DAPT, a γ-secretase inhibitor that indirectly inhibits the Notch signaling pathway, promotes neural differentiation [116, 117]. The VPA was only included for 2 days, and CHIR99021 and DAPT were present from day 3 to day 6. From day 7 to day 8, SAG and Purmorphamine, two agonists for activating the SHH signaling pathway were used to complete the reprogramming process. At day 9, SAG and Purmorphamine were replaced with neurotrophic factors BNDF, NT3, IGF-1, which promote neuronal maturation. To dissect out the contribution of each single molecule toward reprogramming, they removed each molecule from the cocktail and found that removing DAPT, CHIR99021, SB431542, or LDN193189 reduced the astrocyte–neuron conversion dramatically. Removing VPA or SAG and Purmorphamine slightly reduced the efficiency. Removing Thiazovivin or TTNPB did not have a significant effect on the astrocyte–neuron conversion. Another research group reprogrammed human astrocytes into functional neurons with six small molecules (VPA, CHIR99021, Repsox, Forskolin, I-BET151, and ISX9) within 12 days at a conversion efficiency of 8% [118]. Because astrocytes resides in the brain, local delivery of small molecules enabling astrocyte-to-neuron conversion may lead to a direct neuronal conversion of resident astrocytes in situ to meet the ultimate goal of regenerative medicine.

Small molecules induce other cell types

Because of the advantages of small molecules, they have been used to generate many other specialized cells. Lee et al. showed that small molecule Toll-like receptor 3 agonist Poly I: C converted 2% of human fibroblasts into endothelial cells in the presence of endothelial cell growth factors, including VEGF and bFGF [119]. Interestingly, one group using small molecules converted mouse embryonic fibroblasts into three lineage cells simultaneously, including myocytic (10%), glial, or adipocytic (10%) lineage [120]. They used a combination of small molecules (Repsox, Tranylcypromine, CHIR99021, Forskolin) to induce fibroblasts into colonies with diverse morphologies in the same culture. These different colonies could subsequently differentiate into cardiac myocytes, glial cells or adipocytes. This study provides an induction system for generating multi-lineage cells in a culture condition. The efficiency to induce the direct conversion of fibroblasts into hepatocyte-like cells by transcription factors is low (less than 1%), a recent study by screening small molecules promoting this conversion process found that A83-01 and CHIR99021 dramatically enhanced the transcription factor Hnf1α-based transdifferentiation of mouse fibroblasts into hepatocytes (up to 14.5%) [121]. Katsuda et al. used a cocktail of small molecules containing Y-27632, A83-01, and CHIR99021 to dedifferentiate rat and mouse mature hepatocytes into proliferative bipotent liver progenitor cells (with an efficiency of ~25%) that can differentiate into both mature hepatocytes and biliary epithelial cells [122]. Zhu et al. demonstrated that employing non-integrative episomal reprogramming factors in combination with specific growth factors and chemical compounds converted human fibroblasts into pancreatic endodermal progenitor cells (cPE) that can differentiate into functional pancreatic beta-like cells [123]. A brief exposure to 5-Aza also could induce pig dermal fibroblasts to insulin-secreting cells at an efficiency of up to 38% [124]. A transient expression of pluripotency reprogramming factors together with small molecules Activin A and LiCl converted mouse fibroblasts into definitive endoderm-like cells, which were then treated with RA, A83-01, LDE225, and pVc and further converted into the pancreatic progenitor-like cells (PPLCs) (induction efficiency >7%) [125]. The induced PPLCs could mature into cells of all three pancreatic lineages in vivo, including functional insulin-secreting beta-like cells. Using six small molecules SP600125 (JNK inhibitor), SB202190 (p38 inhibitor), Go 6983 (PKC inhibitor), Y-27632 (ROCK inhibitor), PD0325901 (ERK1/2 inhibitor), and CHIR99021 with or without growth factors (TGF-β, bFGF, and LIF), Lai et al. converted human dermal fibroblasts into induced multipotent mesenchymal stem cell-like cells (iMSCs), with an average rate of 38% within 6 days [126]. By removing each small molecule, they decided that three small molecules be sufficient to induce iMSCs, but with a lower efficiency. Functional astrocytes could also be directly generated from mouse fibroblasts with only small compound cocktails (VPA, CHIR99021, Repsox, Tranylcypromine, OAC1) after 25 days [127]. Of these molecules, TGF-β inhibitor Repsox is necessary for astrocytic conversion because removal of Repsox from this combination led to a failure to generate astrocytes.

Conclusion and perspectives

The main approaches for generating iPSCs depend on the overexpression of pluripotency genes, but with low efficiency. With the advancement in dissecting the molecular mechanisms of pluripotency reprogramming, many small molecules have been screened out to improve the reprogramming efficiency or omit exogenous genes. Although

small molecules can improve the transcription factor-based reprogramming, exogenous gene introduction raises clinical safety concerns. Because small molecules minimally affect genome, using small molecules to completely replace exogenous genes is an attractive method for cellular reprogramming. Although the current combination of small molecules can alone induce iPSCs, the efficiency is very low. Future efforts should be focused on finding appropriate chemical cocktails to establish high efficient and safe reprogramming. Nevertheless, potential safety concerns still needs to be addressed for small molecule-based reprogramming, when considering the toxicity of small molecules as drugs and the potential uncontrollable proliferation induced small molecules in clinical therapy.

The differentiation induction of iPSC is often incomplete and low in efficiency. Thus, it is required to control the cell fate of iPSCs specifically. A different combination of signaling pathways can direct the stem cell fate into various cell types. Moreover, the development and differentiation of many cell types often share common pathways. Selectively and dynamically, activating the favorable signaling pathways and inhibiting the other un-appropriate signaling pathways may facilitate the specific differentiation of iPSCs. Using small molecules, it is possible to generate cell types with different degree of differentiation or specific subtype cells such as neuronal subtypes for therapeutic applications.

Complete dedifferentiation of somatic cells generates pluripotent cells, like iPSCs, but acquiring risks of tumor formation. In effect, lower animals like salamander or zebrafish regenerate their body parts through a partial dedifferentiation of resident tissue-specific cells into immature and proliferative cells within their own lineage [128, 129]. This phenomenon suggests that inducing partial dedifferentiation of somatic cells is critical for in vivo regeneration. The cell cycle regulators and proliferation- or differentiation-associated regulators are likely the key targets for small molecules to induce partial dedifferentiation. If resident mature cells in mammals are initiated to undergo partial dedifferentiation by delivery of small molecules, the in situ regeneration will come into true.

Direct conversion of one somatic cell to another type is an attractive alternative for cell-based applications. The use of lineage-specific transcription factors can induce many types of somatic cells or lineage-committed stem/progenitor cells or even many subtypes of one lineage. Based on these induction models, high-throughput and high-definition screening technology should be exploited to determine small molecules that can improve induction models. Small molecules and transcription factor together establish an efficient transdifferentiation without bypassing a pluripotent state, yet introducing exogenous genetic material and altering genome face safety concerns. Thus, it is more desirable to completely replace exogenous genes with small molecules to induce transdifferentiation. Some animals can regenerate their bodies via transdifferentiation mechanisms. For instance, lens regenerates in newts and frogs through transdifferentiation of pigmented epithelial cells or corneal epithelial cells into lens cells [130]. If treatment with small molecules can induce in vivo transdifferentiation of resident cells (such as astrocytes or cardiac fibroblasts) into functional cells that had been lost (such as cardiomyocytes or neurons) during disease development or after injury, non-regenerative organs or tissues in mammals would be expected to gain regenerative power. It is important to mention here that effects of small molecules are not specific for one certain cell type and possibly induce cell fate changes in inappropriate cells, for instance changing or killing the resident normal neurons when injected into the lesion site of the brain. Therefore, it should pay high attention to the side effects when using small molecules to induce transdifferentiation in vivo.

Although small molecules have been beginning to control cell fates, the underlying mechanisms of their effects are not clearly known. Because small molecules often have multiple targets, their actions are nonspecific. In addition, different combinations of small molecules present synergistic or antagonistic effects. It is, therefore, arduous to interpret the action mechanisms of each molecule used in a chemical cocktail. Nevertheless, future studies should further elucidate the mechanisms of small molecules, which will help to assure the safety for their clinical applications or optimize their combinations, concentrations, and exposure time. For clinical use, many works should focus on how to deliver small molecules into the target site in vivo, maintain the functional concentration, and make them only affect a specific cell type. We believe that the development of small molecules will advance the cellular reprogramming and transdifferentiation toward clinical application.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest declared by any of the authors.

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