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Epigenetic Control of Reprogramming and Transdifferentiation by Histone Modifications

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Abstract Somatic cells can be reprogrammed to pluripotent stem cells or transdifferentiate to another lineage cell type. Much efforts have been made to unravel the epigenetic mechanisms underlying the cell fate conversion. Histone modifications as the major epigenetic regulator are implicated in various aspects of reprogramming and transdifferentiation. Here, we discuss the roles of histone modifications on reprogramming and transdifferentiation and hopefully provide new insights into induction and promotion of the cell fate conversion by modulating histone modifications.

Keywords Somatic cells · iPSCs · Reprogramming · Transdifferentiation · Histone modifications · Epigenetic · Cell fate conversion

Introduction

Somatic cells are highly specialized and have a definite molecular pattern that specifies the function and physiology. They are long thought to be stable throughout the lifespan. This dogma, however, is rebutted by the discovery that somatic cells can regress to pluripotent stem cells or directly convert to another lineage cell type. Inducing the somatic state to the pluripotent state, called reprogramming, is initially achieved by somatic cell nuclear transfer (SCNT) experiments in 1958. In these cases, specialized cells from any somatic lineage become pluripotent stem cells after exposing the somatic nucleus to the cytoplasm of the enucleated oocyte [1]. In 2006, overexpressing four pluripotency transcription factors Oct4 (also known as POU5F1), SRY-box2 (Sox2), Krüpple-like factor 4 (Klf4) and c-Myc induces fibroblasts to embryonic stem cell (ESC)-like cells, the so-called induced pluripotent stem cells (iPSCs) [2]. The four factors (Oct4, Sox2, Klf4, and c-Myc) are collectively known as OSKM (or reprogramming factors). Since then, iPSCs have been generated by increasing approaches, including recombinant protein, miRNAs, and small molecules [3, 4]. iPSC generation is a milestone for cell-based regenerative medicine. Like ESCs, iPSCs can differentiate into any cell types required for regeneration. Combined with the genome editing technology such as clustered regularly interspaced short palindromic repeats (CRISPR)-associated Cas9 (CRISPR-Cas9) [5, 6], iPSCs can be used to study gene functions, model diseases, and perform gene therapy. Not only can somatic cells go into the pluripotent state but also directly convert into another lineage, namely transdifferentiation or direct cell conversion [7, 8]. For instance, B cells become macrophages after ectopic expression of master transcription factors, CCAAT-enhancer-binding protein-α (CEBPα) and CEBPβ that are responsible for macrophage commitment from hematopoietic progenitors [9]. The same strategy has converted pancreatic exocrine cells into insulin-producing β-cells [10], and fibroblasts into neurons [11, 12], cardiac cells [13–15], hepatocytes [16], and others. Apart from genetic reintroduction of master transcription factors, several other ways, such as proteins, miRNA, episomal-vectors, and small molecules, are used to induce transdifferentiation [3, 8]. Transdifferentiation provides a more direct way to generate the desired cells, without passing through a pluripotent intermediate.

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The underlying mechanisms of reprogramming and transdifferentiation attract the scientific community. Both processes have to suppress the starting cell-specific genetic program and activate the target cell-specific genetic program. How gene activation and silencing are precisely regulated during the cell fate conversion? Epigenetic modifications including DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs can alter gene expression. They change the chromatin structure from a compact state to a loose state or vice versa, making DNA template accessible or inaccessible to transcription factors and transcriptional machinerіers. Among them, histone modifications are extensively studied during the cell fate conversion. Histone modifications can initiate reprogramming and transdifferentiation, interact with reprogramming factors, regulate expression of key genes, and act as a stimulator or barrier for efficient cell fate conversion. Here, we mainly illustrate the regulatory mechanisms of histone modifications in reprogramming and transdifferentiation.

Histone Modifications: Histone Marks and Histone-Modifying Enzymes

Histones are often subject to post-translational modifications, consisting of methylation, acetylation, phosphorylation, ADP-ribosylation, and ubiquitination, which are executed by histone-modifying enzymes (the histone modifiers) [17]. Distinct histone-modifying enzymes supplement or eliminate an array of covalent modifications to histones. Histone methyltransferases (HMTs) and histone acetyltransferases (HATs) supplement methyl and acetyl groups, whereas histone demethylases (HDMs) and histone deacetylases (HDACs) remove methyl and acetyl groups. Histone modifications have two principal functions: creating global chromatin environments and orchestrating DNA-based biological tasks such as DNA transcription. By partitioning the genome into euchromatin and heterochromatin, histone modifications create global chromatin environments. The active euchromatin generally has high levels of histone acetylation and high methylation at histone H3 of lysine 4 (H3K4), lysine 36 (H3K36), and lysine 79 (H3K79). In contrast, the silent heterochromatin has low levels of histone acetylation and high methylation at histone H3 of lysine 9 (H3K9), lysine 27 (H3K27), and lysine 20 (H4K20). Changing histone modifications can shift the compact chromatin (heterochromatin) to the loose chromatin (euchromatin) (Fig. 1). In addition, histone modifications can orchestrate the ordered recruitment of transcriptional machinery to the DNA sites and regulate DNA transcription. The present review concentrates on roles of histone methylation and acetylation and their histone-modifying enzymes in reprogramming and transdifferentiation.

Histone Modifications Affect the Binding of Reprogramming Factors to the Genome

The canonical reprogramming factors (OSKM) can unlock the pluripotent genetic program in somatic cells, though the reprogramming efficiency is low (<0.1 %) [18, 19]. OSKM bind to their target sites in the genome and reactivate pluripotency genes previously silenced [20], suggesting that accessing the chromatin by OSKM is a prerequisite for efficient reprogramming. The low efficiency indicates that the chromatic state of somatic cells is resistant to OSKM binding.

Heterochromatin enriched with repressive histone marks inhibits the binding of reprogramming factors. Although Oct4, Sox2, and Klf4 (OSK) can approach some closed chromatic regions that are absent of evident histone marks, many regulatory regions of genes bound by OSK in the later pluripotent state are indeed not occupied in the early stage, suggesting that many regions within the genome are inhibitory to their binding [20–22]. Megabase-scale heterochromatic domains deposited by the repressive H3K9 trimethylation (H3K9me3) in fibroblasts resist OSKM binding [20]. These H3K9me3-enriched heterochromatins contain enormous genes required for pluripotency such as Nanog, Dppa4 (developmental pluripotency associated 4), Sox2, Gdf3 (growth differentiation factor 3), and Prdm14 (PR domain containing 14) [20, 23]. These genes will not be activated until the later phases of reprogramming [23, 24], which may partially explain why pluripotency genes are more refractory to be activated than others are. Inhibiting H3K9me3 enhances iPSC generation, demonstrating that H3K9me3 heterochromatic domains impede reprogramming of somatic cells to pluripotency [20]. Similar H3K9me3-enriched heterochromatic domains (called reprogramming resistant regions, RRRs) observed in SCNT-mediated reprogramming also inhibit reprogramming [25]. Therefore, the widespread H3K9me3 mark in somatic cells impedes reprogramming factor binding and acts as a critical epigenetic barrier for efficient reprogramming.

Reprogramming Factors Change Histone Modifications during Reprogramming

Although many genomic sites in somatic cells are resistant to reprogramming factor binding, the initial expression of reprogramming factors can trigger concerted genome-wide changes in histone modifications and chromatin states, thereby suppressing somatic genes and activating pluripotency genes (Fig. 2). In OSKM-transfected cells that have undergone several cell divisions, active H3K4 dimethylation (H3K4me2) is redistributed at more than a thousand loci prior to their transcriptional activation. These target loci contain promoters and enhancers of many pluripotency-related genes

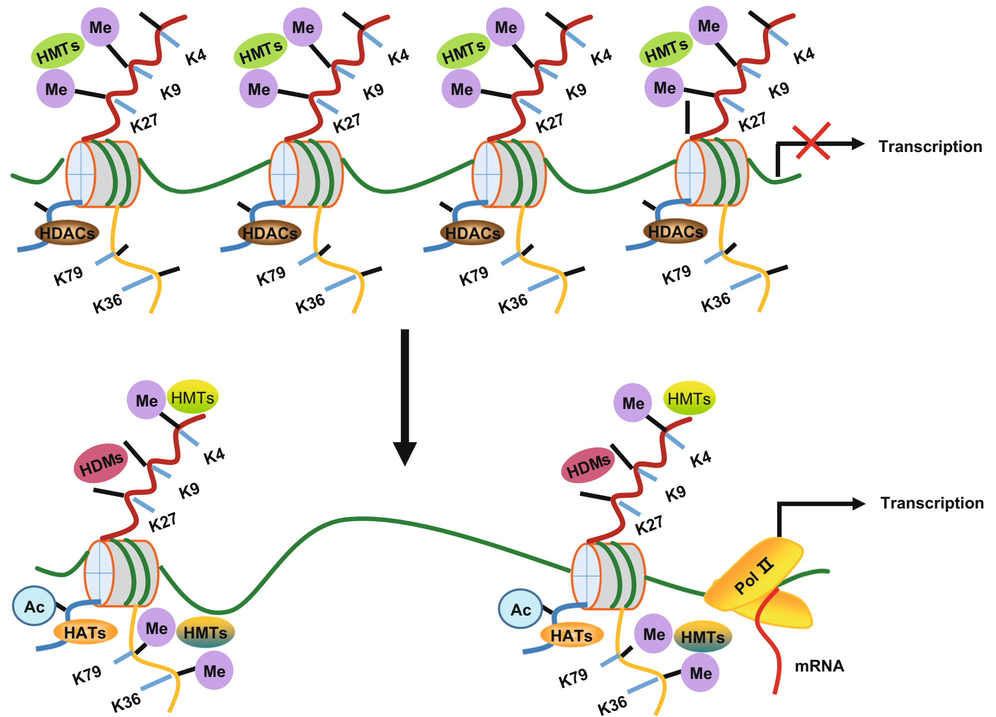

Fig. 1 A model of histone modifications in regulating gene transcription. Compact chromatins are enriched with high levels of repressive histone marks such as methylated (Me) lysine 27, lysine 9 of histone 3 (H3K27, H3K9) and low levels of active histone marks such as methylated lysine 4, lysine 36, lysine 79 of histone 3 (H3K4, H3K36, H3K79) and low levels of histone acetylation. By contrast, when methylated H3K27 and H3K9 are removed by histone demethylases (HDMs), H3K4, H3K36, and H3K79 are methylated by histone methyltransferases (HMTs), and histone is acetylated by histone acetylases (HATs), compact chromatins are changed into loose chromatins, facilitating gene transcription. HDACs, histone deacetylases (HDACs)

and developmentally regulated genes, which will not be activated until later stages of iPSC formation [26]. By contrast, H3K4 trimethylation (H3K4me3) only changes locally [26, 27]. Distinct two increase phases of H3K4me3 levels are detected; the early increase phase is associated with activation of some ESC-associated genes, and the later increase phase relates to reprogramming to pluripotency [27]. The repressive trimethylated H3K27 (H3K27me3) decreases early after ectopic reprogramming factor expression, creating a transient, open chromatin state [26–28].

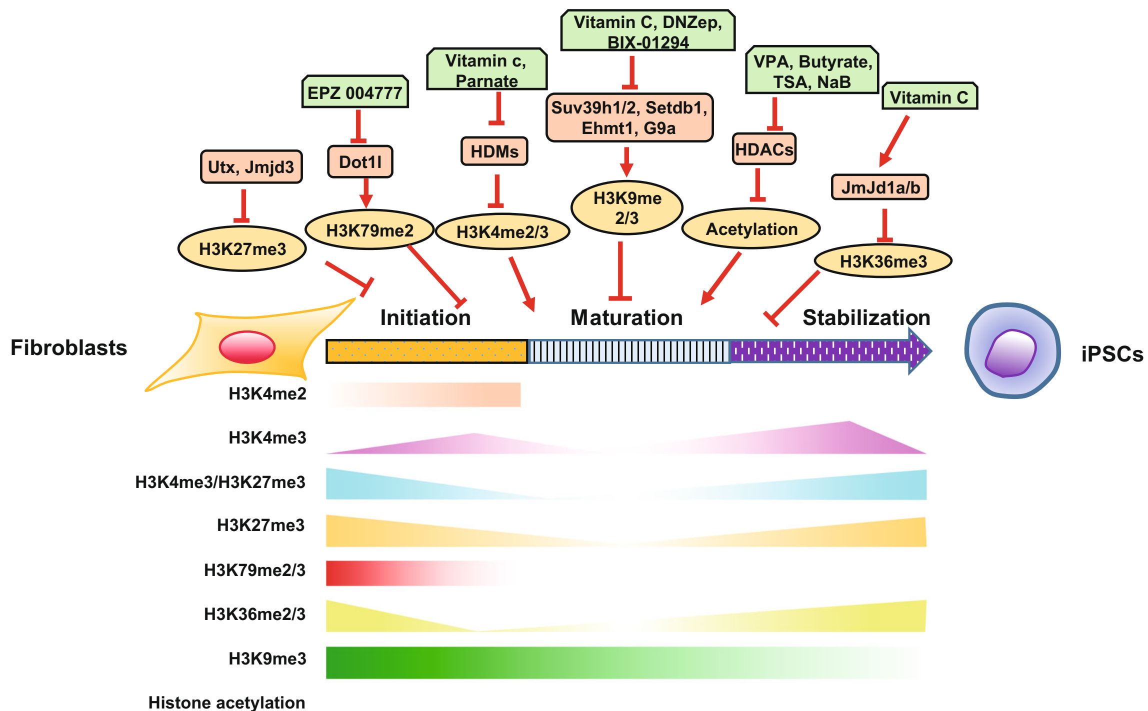

Fig. 2 Histone modifications change during reprogramming. The colored bars represent the dynamic changes in histone modifications during iPSC generation. The yellow ellipse indicates histone marks that inhibit or promote reprogramming, and light red rectangular indicates the corresponding histone-modifying enzymes and small molecules that regulate reprogramming

Changes in histone modifications influence gene expression and reprogramming. Gain of active histone marks and loss of repressive histone marks facilitate the expression of pluripotency genes; conversely, loss of active histone marks and gain of repressive histone marks silence the somatic cell genes. For example, pluripotency-associated genes are activated following the loss of H3K27me3 and acquisition of H3K4me3 [24, 29]; the fibroblast-specific gene Pdgfrb (platelet derived growth factor receptor beta) at the initial stage of reprogramming is gradually silenced with early loss of active H3K4me3 and subsequent acquisition of repressive H3K27me3 [24]. Of note, repressive H3K27me3 has dual roles in reprogramming. H3K27me3 depletion increases expression of pluripotency genes, and maintenance or de novo acquisition of H3K27me3 at some fibroblast-specific gene sites silences these somatic genes during reprogramming [30]. Decreasing active histone marks at somatic cell genes contributes to reprogramming. Fibroblast-specific genes associated with the epithelial to mesenchymal transition (EMT), such as snail family transcriptional repressor 2 (Snail2), transforming growth factor beta 2 (Tgf-β2), and transforming growth factor beta receptor 1 (Tgfbr1), lose the active H3K79 dimethylation (H3K79me2) at the early phase of reprogramming, corresponding with silencing of EMT-associated genes [31]. Thus, the kinetic of repressing somatic cell genes and activating pluripotency-associated genes during reprogramming is partly determined by histone modifications.

Reprogramming Factors Interact with Histone-Modifying Enzymes to Regulate Reprogramming

How do reprogramming factors alter biochemically histone modifications? Reprogramming factors can interact with histone-modifying enzymes to reset the epigenome during reprogramming (Fig. 3). For instance, WD repeat domain 5 (Wdr5), a core component of the mammalian Trithorax group (TrxG) complex trimethylating H3K4, is recruited by Oct4 to maintain H3K4me3 at promoters of self-renewal-associated genes in mouse ESCs [32]. Wrd5 expression levels is upregulated during iPSC generation, and depleting Wrd5 at the initial reprogramming stages reduces the efficiency of iPSC generation. Oct4 likely recruits Wrd5 to re-establish the H3K4me3 high chromatin signature characteristic of ESCs in fibroblasts [32]. Moreover, Oct4 recruits histone demethylase Jmjd1c (jumonji domain containing 1c) to the Oct4 enhancers and other Oct4 targets such as Nanog promoters, leading to H3K9me2 reduction and endogenous Oct4 expression in ESCs. Reprogramming factors can upregulate Jmjd1c expression, but Jmjd1c knockout diminishes endogenous Oct4 expression and causes incomplete reprogramming, proving that the interaction between Oct4 and Jmjd1c is required for complete iPSC generation [33]. Hanna and colleagues demonstrated that Oct4, Sox2, and Klf4 engage with the histone demethylase Utx to demethylate the H3K27me3 at promoters of the pluripotency-associated genes [29]. These discoveries may interpret why Oct4 cannot be replaced or omitted during reprogramming [34–36], possibly because Oct4 can cooperate with histone modifiers to reset the somatic epigenome. Like Oct4, c-Myc is capable of collaborating with histone modifiers. c-Myc can induce the expression of two core subunits (Wrd5 and Dpy30) of the Set1/Mll family of H3K4 methyltransferase complexes by binding to their promoters during reprogramming and physically interact with Wrd5 and Ash2l (another core subunit of the Set1/Mll complex) [37]. In addition, c-Myc can stimulate the expression of the SAGA histone acetyltransferase complex, in particular, GCN5; c-Myc and GCN5 cooperate to form a positive forward loop and activate a network of RNA processing genes during the initial reprogramming [38]. Rao et al. suggested that c-Myc interact with the H3K27me3 methyltransferase Ezh2 to deposit H3K27me3 at the pro-EMT gene Tgfbr2 (transforming growth factor beta receptor 2) and represses its expression. Consequently, c-Myc and Ezh2 together inhibit the pro-EMT TGF-β signaling and in turn promote MET during reprogramming [39]. These studies conclude that reprogramming factors can cooperate with histone-modifying enzymes to change histone marks and chromatin landscape during reprogramming.

Genetically Modulating Histone-Modifying Enzymes Promotes Reprogramming

Since repressive histone modifications block reprogramming, modulating histone modifications by targeting the histone-modifying enzymes may advance reprogramming (Fig. 2 and Table 1). H3K9me2/3 marks are epigenetic roadblocks for reprogramming [20, 40, 41], whose levels are controlled by the balance between H3K9 methyltransferases (Suv39h1/2, Setdb1, Ehmt1, and G9a) and H3K9 demethylases (Jmjd1a, Jmjd1b, Jmjd1c, Jmjd2d). Targeting these enzymes potentially promotes reprogramming. Knocking down H3K9 methyltransferases (Suv39h1/2, Setb1, Ehmt1, and G9a) accelerates iPSC generation [20, 31, 40, 41]. Depleting Suv39h1/2 in donor cells or ectopically expressing H3K9 demethylase Jmjd2d (or Kdm4d) in oocytes enhances SCNT-mediated reprogramming [25].

The H3K27me3 histone mark regularly suppresses transcriptional activation, though inhibiting H3K27me3 methyltransferases diminishes reprogramming. Several studies have consistently found that inhibiting H3K27me3 methyltransferases such as Polycomb repressive complex 1 (PRC1) subunits (Bmi1 and Ring1) and Polycomb repressive complex 2 (PRC2) subunits (Ezh2, Eed, Suz1, Jarid2, Mtf2, and esPRC2p48) impairs the reprogramming efficiency [30, 31, 42]. Overexpressing the H3K27me3 methyltransferase PRC1

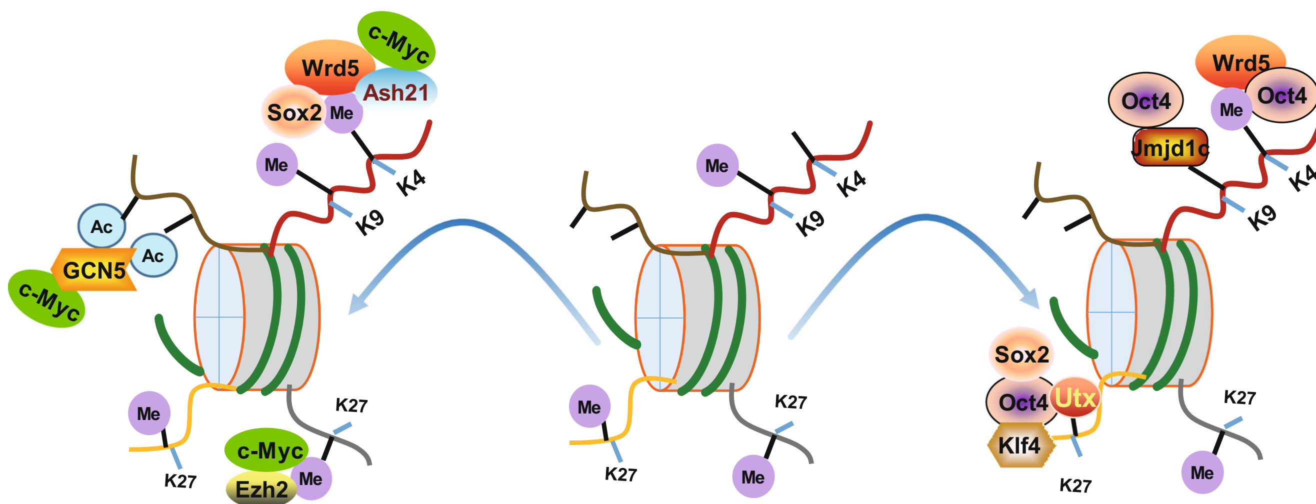

Fig. 3 Interaction between reprogramming factors and histone-modifying enzymes during iPSC generation. The right diagram mainly illustrates Oct4-dependent interaction with histone-modifying enzymes to regulate histone modifications at pluripotency-related loci. Oct4 can recruit H3K4 methyltransferase Wrd5 to methylate H3K4 and recruit histone demethylase Jmjd1c to demethylate H3K9me2 at pluripotency genes, resulting in their transcriptional activation. Oct4, Sox2, and Klf4 can interact with H3K27me3 demethylase Utx to reduce H3K27me3 at pluripotency genes, leading to their expression. The left diagram describes the c-Myc-dependent interaction with histone-modifying enzymes. c-Myc can interact with Wrd5 and Ash21, the core subunits of the Set1/Mll family of H3K4 methyltransferases, adding active H3K4me3. In addition, c-Myc recruits histone acetyltransferase complex GCN5 to activate a network of RNA processing genes during reprogramming, and interact with H3K27me3 methyltransferase Ezh2 to deposit H3K27me3 at pro-EMT genes and suppress their expression during reprogramming

subunit Bmi1 promotes reprogramming [43], and overexpressing PRC1 subunit Ring1b and PRC2 subunit Ezh2 facilitates the ESC fusion-mediated reprogramming [44]. That is because H3K27me3 can silence somatic cell genes [29]. Reducing H3K27me3 globally by knocking out its histone methyltransferases will inevitably delay or block the loss of somatic cell genes during reprogramming, damaging the reprogramming efficiency. Increasing the global H3K27me3 levels by inhibiting H3K27me3 demethylase Utx blocks reprogramming [29], while inhibiting another H3K27me3 demethylase Jmjd3 promotes reprogramming [45]. What causes the contrasting outcomes? H3K27me3 possibly represses both pluripotency genes and somatic genes; therefore, globally increasing or decreasing H3K27me3 will weaken reprogramming. Alternatively, distinct H3K27me3 demethylases function differently; one type of H3K27me3 demethylase may mainly remove H3K27me3 from the pluripotent loci, and another one may remove H3K27me3 from the somatic loci.

Table 1 Genetic modulation of histone-modifying enzymes during reprogramming

Category	Subfamily	Gain of function Effects on reprogramming	Loss of function	Ref
H3K9 methyltransferases	Suv39h1/2	↓	↑	[20, 31, 40]
	Setb1		↑	[40, 41]
	Ehmt1		↑	[41]
	G9a (Ehmt2)		↑	[40, 41]
H3K9 demethylases	Kdm4b (Jmjd2b)	↑		[40]
	Kdm3a (Jmjd1a)		↓	[40]
	Kdm3b (Jmjd1b)		↓	[40]
	Kdm3c (Jmjd1c)		↓	[40]
H3K27 methyltransferases	PRC1(Bmi1, Ring1)	↑	↓	[31, 44]
	PRC2(Ezh2, Eed, Suz1)	↑	↓	[23, 30, 31, 44]
H3K27 demethylases	Utx		↓	[29]
	Jmjd3 (Kdm6b)	↓	↑	[45]
H3K79 methyltransferases	Dot1l		↑	[31]

PRC1 Polycomb repressive complex 1, *PRC2* Polycomb repressive complex 2

H3K79me2 is generally related to gene expression, yet decreasing H3K79me2 by inhibiting the H3K79me2 methyltransferase Dot1l replaces some reprogramming factors (Klf4 and c-Myc) to induce reprogramming after ectopic expression of only Oct4 and Sox2 [31]. Moreover, Dot1l depletion upregulates other two pluripotency-associated transcription factors (Nanog and Lin28) and reduces H3K79me2 in fibroblast-specific genes associated with EMT [31]. These results show that decreasing H3K79me2 at EMT-associated genes can promote reprogramming. Hence, regulating the expression of histone-modifying enzymes may speed up the epigenetic changes and potently improve the reprogramming efficiency.

Small Molecules Targeting Histone Modifications Promote Reprogramming

Genetically manipulating histone-modifying enzymes is often global and irreversible, so changing them locally or temporarily is better and safer. Small chemical compounds are available to inhibit or activate histone-modifying enzymes. These small molecules have advantages over the genetic manipulation, because they are cell permeable, nonimmunogenic, more cost-effective, and more easily synthesized, preserved, and standardized. More importantly, they inhibit and activate the enzyme activity reversibly and finely only by varying the time and concentrations [46]. Many small molecules are used to induce and improve reprogramming [46, 47] (Table 2).

Reducing the repressive histone modifications by small molecules can enhance reprogramming considerably. Vitamin C treatment enhances the reprogramming of murine and human fibroblasts to pluripotency [48], partly because it can increase the expression of several H3K9 demethylases, which then demethylate H3K9me2/3 and shift the heterochromatic state surrounding pluripotency genes to euchromatic state [40, 49]. 3-deazaneplanocin A (DNZep), an S-adenosylhomocysteine (SAH) hydrolase inhibitor, can reduce the H3K9me3 deposition at the Oct4 promoter and enhance reprogramming [50]. Chemical drug CYT296, when added to reprogramming medium, disrupts compact chromatins and reduces heterochromatin protein 1alpha (HP1alpha) and H3K9me3, increasing the OSKM- and Oct4-mediated reprogramming [51]. BIX-01294, known as a specific inhibitor of H3K9me3 methyltransferase G9a, is used to speed up the OK (Oct4 and Klf4)-mediated reprogramming of the mouse embryonic fibroblasts [35] and neural progenitor cells (NPC) [52] into iPSCs or the SCNT-medicated reprogramming [53]. Small molecules can also change other repressive histone marks. Vitamin C treatment decreases H3K27me3 at promoter regions of pluripotency genes such as Zfp42 (ZFP42 zinc finger protein), Ddx4 (DEAD-box helicase 4), and Nanog during the transition of pre-iPSCs to iPSCs [54]. Because H3K79 methylation inhibits reprogramming, decreasing H3K79 methylation with EPZ 004777, an inhibitor of H3K79 histone methyltransferase Dot1l, improves the reprogramming rate [31, 55].

As active histone marks favor gene activation, enhancing the active H3K4 methylation by small molecules facilitates reprogramming. Hochedlinger and colleagues found that vitamin C treatment induces a full reprogramming of B-lymphocytes into iPSCs that can generate entirely iPSC-derived mice ("all iPSC-derived mice"), which is not observed without vitamin C treatment. Why can vitamin C improve the reprogramming quality? The imprinted Dlk-Dio3 locus is lost in the majority of iPSC lines, whose loss impairs the potential of iPSCs to generate all iPSC-derived mice. Vitamin C, however, can preserve the imprinted Dlk-Dio3 locus by maintaining H3K4me2 and regaining H3K4me3 on the promoter [56]. Likewise, maintaining the methylated H3K4 by an H3K4 demethylase inhibitor, parnate, boosts OSK-or Oct4-mediated iPSC generation from fibroblasts [36], or OK-mediated iPSC generation from keratinocytes [57]. Interestingly, reducing H3K4me3 methylation by inhibiting the histone H3K4 methyltransferase MLL1 activity with small-molecule inhibitor MM-401 induces mouse epiblast stem cells to naïve pluripotency efficiently [58]. In the case, blocking MLL1 redistributes globally the repressive H3K4 monomethylation (H3K4me1) at enhancers of lineage determinant factors and markers of epiblast stem cells, subsequently repressing their expression.

Although H3K36 di−/tri-methylation (H3K36me2/3) marks are usually transcriptional active, inhibiting H3K36me2/3 by vitamin C increases iPSC production [59]. Vitamin C upregulates the levels of the H3K36 demethylases Jhdm1a/1b, which then reduce H3K36me2/3. Specifically, Jhdm1b removes active H3K36me2/3 marks from the Ink4/Arf locus required for cell senescence, therefore, repressing the Ink4/Arf-mediated cell senescence and promoting fibroblast proliferation during reprogramming [59]. This study indicates that removing active histone marks from genes inhibiting reprogramming promotes reprogramming potently.

Histone acetylation generally correlates with gene activation. Increasing the level of histone acetylation by HDAC inhibitors seems to enhance reprogramming. Valproic acid (VPA), an HDAC inhibitor, enables efficient reprogramming of fibroblasts in the absence of c-Myc [60] or both c-Myc and Klf4 [34]. Sodium butyrate (NaB), a naturally occurring fatty acid, is an HDAC inhibitor; when added into the induction medium, it increases the generation of iPSCs [61]. Other HDAC inhibitors such as trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA), display similar promoting effects [60–62]. Pandian and colleagues developed a small-molecule SAHA-PIP comprising of SAHA and the sequence-specific DNA binding pyrrole-imidazole polyamides (PIP) and found this molecule able to induce genome-wide epigenetic reprogramming and to activate Oct-3/4 regulated pluripotency genes in human

Table 2 Small molecule-mediated modulation of histone-modifying enzymes during reprogramming

Small molecules	Targeted histone-modifying enzymes	Effects on reprogramming	Ref
Vitamin C	H3K9 methyltransferases	Promotes OSKM-mediated reprogramming	[40]
	H3K9 demethylases	Promotes OSKM-mediated or vitamin C + 2i-mediated reprogramming	[40, 49]
	H3K27?	Promotes reprogramming of pre-iPSCs into iPSCs	[54]
	H3K4?	Promotes reprogramming of B lymphocytes into iPSCs	[56]
	H3K36 demethylases	Promotes OSK-mediated reprogramming	[59]
DNZep	H3K9 methyltransferases	Promotes small molecule-mediated reprogramming	[50]
CYT296	H3K9?	Promotes OSKM- or Oct4-mediated reprogramming	[51]
BIX-01294	H3K9 methyltransferases	Promotes OK- or SCNT-mediated reprogramming	[35, 52, 53]
EPZ 004777	H3K79 methyltransferases	Promotes OSKM-mediated or small molecule-mediated reprogramming	[31] [65]
Parnate	H3K4 demethylases	Promotes OSK- or Oct4-mediated reprogramming of fibroblasts, or OK-mediated reprogramming of keratinocytes	[36, 57]
MM-401	H3K4 methyltransferases	Promotes reprogramming of epiblasts to naïve pluripotent state	[58]
Valproic acid (VPA)	HDACs	Promotes OSK- or OS-mediated reprogramming	[34, 60]
Sodium butyrate	HDACs	Promotes OSKM-, OSM-, or OSK-mediated reprogramming	[61]
Trichostatin A (TSA)	HDACs	Promotes OSK- or OS-mediated reprogramming	[60]
SAHA-PIP	HDACs	Promotes reprogramming	[63]
RSC133	HDACs	Promotes reprogramming	[64]

? refers to methyltransferases or demethylases; OSKM, Oct4, Sox2, Klf4, c-Myc; OSK, Oct4, Sox2, Klf4; OK, Oct4, Klf4; OSM, Oct4, Sox2, c-Myc; OS, Oct4, Sox2; HDACs, histone deacetylases; SAHA-PIP, suberoylanilide hydroxamic acid-pyrrole-imidazole polyamides

fibroblasts [63]. RSC133, a new synthetic derivative of indoleacrylic acid/indolepropionic acid, can dually inhibit histone deacetylase and DNA methyltransferase; when administered, it can remarkably accelerate the reprogramming to pluripotency [64].

Histone Modification Changes during Transdifferentiation

Transdifferentiation frequently occurs between various somatic cells. By ectopically expressing reprogramming factors or master regulators or treating with small molecues, somatic cells (especially skin fibroblasts) can convert into many other cells such as cardiomyocytes, neural cells, and others. The conversion process is accompanied by dramatic changes in histone modifications and gene expressions (Fig. 4).

Transdifferentiation of Fibroblasts into Cardiomyocytes Fibroblasts can be induced to cardiomyocytes by several strategies. For the reprogramming factor-mediated direct conversion, early transient expression of OSKM combined with the cardiac culture medium induces fibroblasts into cardiomyocytes. The initial expression of reprogramming factors increases H3K4me3 and decreases H3K27me3 at the promoter of endogenous Oct4. The cardiac specific genes, actinin α2 (Actn2), ryanodine receptor 2 (Ryr2), troponin T2 (Tnnt2), which are expressed at the later stages of the cardiac development, gain H3K4me3 and lose H3K27me3 at the later transdifferentiation stage [66]. In lineage factor-mediated direct conversion model, ectopic expression of three cardiac master transcription factors, GATA binding protein 4 (Gata4), myocyte-specific enhancer factor 2C (Mef2c), and T-box 5 (Tbx5) (GMT) forces fibroblasts into cardiomyocytes. At the early stage but not the later stage in this model, the repressive H3K27me3 is lost at the promoters of cardiac specific genes, including the cardiac transcription factors (GMT), cardiac structural genes such as Tnnt2, α-myosin heavy chain (Myh6), and functional genes such as Ryr2, phospholamban (Pln), and natriuretic peptide precursor type A (Nppa), and active H3K4me3 is enriched at these promoter regions [13, 67]. By contrast, H3K4me3 mark is gradually lost at fibroblast-specific genes, and at the later stage, the H3K27me3 is increased at these loci [67]. These indicate that histone modification changes induced by cardiac master transcription factors bring about an early rapid activation of cardiac gene program and a later progressive suppression of fibroblast fate. Another study converting fibroblasts into cardiomyocytes by transfection with Gata4, Mef2c, Tbx5, and Hand2 (GMTH) showed that H3K4me2 peaks change from a fibroblast state to a cardiomyocyte state. Especially, the H3K4me2 levels are increased in the muscle-specific miRNAs, miR-1-2/miR-133a-1 cluster that is crucial for cardiac muscle cell differentiation [65]. The two approaches are different in the sequence to activate cardiac-specific genes. It seems to suggest that ectopic expression of cardiac master transcription factors is more prone to establish histone modifications favoring cardiac gene expression as compared to reprogramming factors. Accordingly,

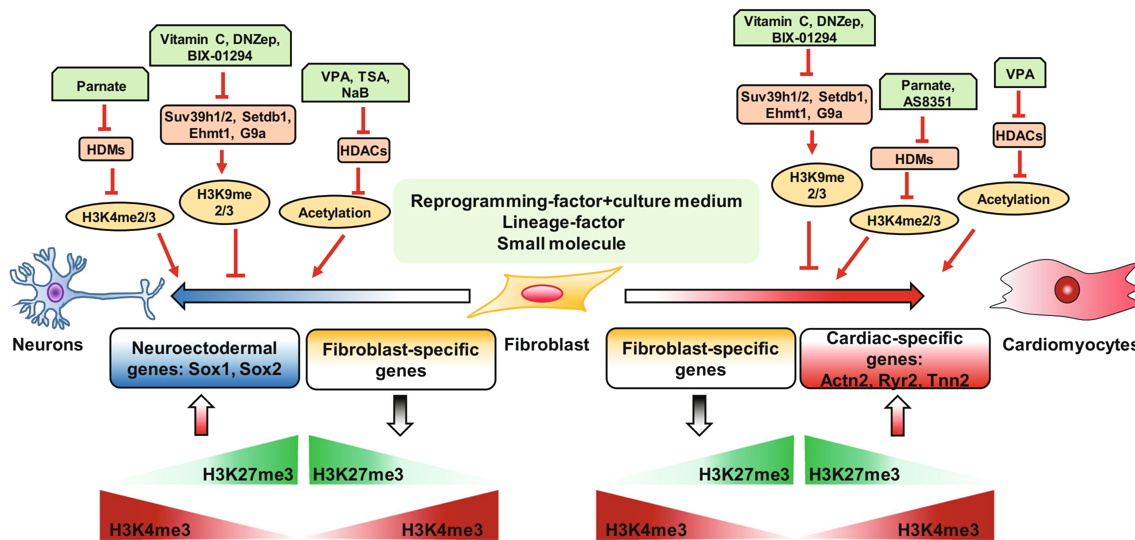

Fig. 4 The changes in gene transcription and histone modification during transdifferentiation. Somatic cells (like fibroblasts) can transdifferentiate into another lineage cell type such as cardiomyocytes and neurons. During the transdifferentiation, fibroblast-specific genes are downregulated while the target cell-specific genes (such as cardiac- or neural-specific genes are up-regulated. In general, reduced H3K27me3 and increased H3K4me3 are associated with expression of cardiac-specific or neural-specific genes, and increased H3K27me3 and reduced H3K4me3 are associated with silencing of fibroblast-specific genes. Light red rectangular indicates the corresponding histone-modifying enzymes and small molecules that regulate reprogramming

lineage-restricted transcription factors shift the epigenetic state of somatic cells to that of target cells more directly.

Great changes in histone modifications occur in the small molecule-mediated direct conversion of fibroblasts to cardiomyocytes. In a conversion induced by nine small molecules, the heterochromatic foci containing H3K9me3 are reduced, and loss of H3K27me3 and gain of H3K4me3 are observed in a subset of genomic loci associated with the developmental process and cell differentiation. Conversely, some cardiac developmental genes gain H3K4me3 and H3K27 acetylation (H3K27Ac) and lose H3K27me3 [68]. Another combination of small molecules also converts fibroblasts to cardiomyocytes, accompanied by a decreased H3K27me3 and an increased H3K4me3 in the promoter regions of cardiac specific genes (Actn2, Ryr2, Tnnt2) [69].

Transdifferentiation of Fibroblasts to Neural Cells Neurons can directly derive from fibroblasts (Fig. 4). For example, transient expression of OSKM together with appropriate neural induction medium generates neural progenitor cells (NPCs) from fibroblasts [70]. In the case, the promoter region of SRY-box 1 (Sox1), the earliest neuroectodermal marker, undergoes an early loss of H3K27me3 from day 4 onward and a later gain of H3K4me3 from day 8. By contrast, the Oct4 promoter in the induced fibroblasts is permanently marked by H3K27me3, resembling adult NPCs. This suggests generating NPCs from fibroblasts does not involve the transition to pluripotency. In addition, neural stem cells can arise from fibroblasts by small molecules. Zhang and colleagues used nine small molecules to derive neural stem cells from fibroblasts. At the early stage, small molecules trigger a loss of H3K27me3 and a gain of H3K4me3 at the promoter regions of the ectoderm development genes. Specifically, Sox2 locus gradually gains H3K4me3 and H3K27Ac and lose H3K27me3, while the promoter regions of fibroblast-associated genes gradually lose H3K4me3 and gain H3K27me3 [71]. The changes in the histone modification pattern support a fibroblast-neural fate transition.

Transdifferentiation of Fibroblasts into Other Cells Overexpressing two epidermal development- and differentiation-associated transcription factors, p63 and Klf4, induces human fibroblasts into keratinocyte-like cells efficiently and rapidly [72]. After induction, fibroblasts share similar epigenetic signature with primary keratinocyte cells. Induced fibroblasts lose the repressive H3K27me3 mark and gain the active H3K4me3 on the promoter of keratinocyte-specific genes, keratin 14 (Krt14), gap junction protein beta 2 (Gjb2), and gap junction protein beta 3(Gjb3). Treatment with toll-like receptor 3 agonist and endothelial cell (EC) growth factors transdifferentiates human fibroblasts into ECs. The induced ECs show an increased H3K4me3 deposition and a decreased H3K27me3 deposition at the promoter of EC marker CD31 as compared with fibroblasts [73].

Histone-Modifying Enzymes Regulate Transdifferentiation

The well-established histone-modifying enzymes in somatic cells maintain the differentiated state and prevent somatic cell transdifferentiation. Changes in these histone modifiers are

closely linked to transdifferentiation (Fig. 4 and Table 3). Induction of cardiomyocytes from fibroblasts by ectopic expression of cardiac master factors has low efficiency, which may be ascribed to epigenetic barriers. Zhou and colleagues demonstrate that the epigenetic regulator, Bmi1, a polycomb group protein, blocks the conversion of fibroblasts into cardiomyocytes [74]. They deplete Bmi1 and then increase cardiomyocyte induction from fibroblasts. Bmi1 depletion increases the levels of active H3K4me3 and reduces levels of repressive monoubiquitylation of lysine 119 on histone H2A (H2AK119ub) at cardiogenic genes, subsequently derepressing cardiogenic genes in fibroblasts. Histone deacetylase HDAC7 is highly expressed in pre-B cells, and but reduced during CEBPα-mediated conversion of pre-B cell into macrophages [75]. In pre-B cells, HDAC7 expression silences the transcription program characteristic of macrophages; HDAC7 inhibition thus depresses macrophage-associated genes and favors macrophage induction.

Somatic cells can transdifferentiate naturally with high efficiency, in addition to experimental induction, and histone modifiers participate in the natural transdifferentiation. Naturally occurring transdifferentiation contributes to tissue and organ development and regeneration and even disease development. At the larval stage, *Caenorhabditis elegans* postmitotic rectal epithelial cell, Y cell, can naturally convert into motor neuron, PDA, robustly. The natural conversion requires the activity of H3K27me3/me2 demethylase, Jmjd-3.1 and H3K4 methyltransferase Set1 complex [76].

Some animals regenerate highly by somatic cell transdifferentiation. One classic example is the lens regeneration in newts. When removed, the newt lens can grow a new one by transdifferentiation of the dorsal iris pigmented epithelial cells (PECs) into lens cells. The transdifferentiation process involves an initial dedifferentiation of PECs into stem-like cells and a later differentiation to lens cells. During the initial dedifferentiation step, H3K4me3 and H4 acetylation (H4Ac) are upregulated, and the H3K9 acetylation (H3K9Ac) is decreased, with no significant changes in H3K9me2/3 [77]. Correspondingly, several types of histone acetyltransferases (CREB binding protein/p300, MYST3), deacetylases (HDAC2, HDAC5), and demethylases (Jmjd2, Jmjd1b) change in this step [78]. However, H3K27me3 does not change in dorsal iris PECs but increases in ventral iris. This dorsal-ventral difference may partly explain why ventral iris PECs can not transdifferentiate into lens cells during lens regeneration [77]. We hypothesize that H3K27me3 methyltransferases are likely to increase in the ventral iris, while H3K27me3 methyltransferases and demethylases keep a good balance in the dorsal iris that permits transdifferentiation, but the hypothesis is required for future verifying. Whether the histone-modifying enzymes are implicated in the later, selective differentiation to lens cells remains unclear.

Aberrant transdifferentiation also leads to disease development. Transdifferentiation of hepatic stellate cells (HSCs) to myofibroblasts contributes to liver fibrosis. The peroxisome proliferator-activated receptor-gamma (PPARγ) gene inhibits the conversion of HSCs into myofibroblasts. However, PPARγ gene is transcriptionally silenced during liver fibrosis, associated with myofibroblast transdifferentiation. Methyl-CpG binding protein 2 (MeCP2) is found to promote H3K9 methylation at the 5′ end of of PPARγ and to increase histone methyltransferase EZH2 expression and H3K27 methylation at the 3′ exons of PPARγ, subsequently repressing PPARγ [79]. In addition, various H3K4 histone methyltransferases (MLL1, MLL5, Set1, and ASH1) are upregulated in the conversion of HSCs into myofibroblasts, consistent with increased levels of active H3K4me3 [80]. Among them, ASH1 is a direct component of MeCP2-dependent epigenetic pathway and directly binds to the promoter regions of some pro-fibrogenic gene, such as α smooth muscle actin (αSMA), collagen I, tissue inhibitor of metalloproteinase-1 (TIMP1), and TGFβ1, and increases H3K4me3 at their promoter regions. It is reasonable to speculate that the normally low levels of these histone methyltransferases in livers prevent transdifferentiation of HSCs and liver fibrosis.

Histone-modifying enzymes play crucial roles in experimental and natural transdifferentiation. As the

Table 3 Genetic modulation of histone-modifying enzymes during transdifferentiation

Enzymes	Subfamily	Effects on transdifferentiation	Ref
H3K4 methyltransferases	Bmi1	Bmil depletion enhances conversion of fibroblasts into cardiomyocytes	[74]
	ASH1	Promotes transdifferentiation of HSCs into myofibroblast-like cells	[80]
	Set1	Promotes transdifferentiation of *Caenorhabditis elegans* rectal epithelial cell into motor neurons	[76]
Histone deacetylases	HDAC7	HDAC7 reduction promotes conversion of pre-B cells into macrophages	[75]
H3K27 demethylase	Jmjd-3.1	Promotes transdifferentiation of Caenorhabditis elegans rectal epithelial cell into motor neurons	[76]
	EZH2	Promotes transdifferentiation of HSCs into myofibroblast-like cells by suppressing PPARγ	[79]

ASH1 Absent, small, or homeotic disc 1, *HDAC7* Histone deacetylase 7, *HSCs* Hepatic stellate cells, *PPARγ* peroxisome proliferator-activated receptor-gamma

executioners of histone modifications, they may control the transdifferentiation process fundamentally. In vitro, adjusting histone-modifying enzymes may help to improve inducing new cells. Spontaneous changes of these enzymes during development may control the cell fate commitment. Tissue injuries shift these enzymes to favor conversion of somatic cells near to injury sites to another appropriate cells required for tissue repair and regeneration, for instance, lens regeneration. Alternatively, tissue injures may shift these enzymes to prefer conversion of somatic cells to fibroblast-like cells, resulting in fibrosis. Modulating these enzymes therefore will promote regeneration and inhibit fibrosis.

Small Molecules Targeting Histone Modifications Promote Transdifferentiation

As histone modifications regulate transdifferentiation, modulating histone modifications has turned out to be an effective way to induce and improve transdifferentiation. Small molecules regulating histone modifiers have been widely used to reinforce the transcription factor-mediated direct conversion or induce cell conversion combined with other chemical drugs targeting signaling pathways in the absence of transcription factors (Table 4).

Several small molecues are screened to enhance the transcription factor-mediated transdifferentiation. Treatment with a combination of small molecules that inhibit HDACs, H3K27 methyltransferases, and H3K4me2 demethylases accelerates the direct conversion of fibroblasts into neural-precursor-like cells induced by ectopic expression of Pax6 and Foxg1 [81]. The Oct4-induced conversion of fibroblasts to cardiomyocytes is enhanced by parnate, an inhibitor of the lysine-specific demethylase 1 (LSD1) that demethylates H3K4me3 [82].

Transdifferentiation can be induced only by small molecules targeting histone modifications and signaling pathways. Differentiated pancreatic α cell possesses many more genes marked by bivalent H3K4me3/H3K27me3 marks compared with pancreatic β cell, implying that controlling the histone methylation level probably converts pancreatic α cell into β cell. Bramswig and colleagues treated cultured pancreatic islets with an unspecific histone methyltransferase inhibitor Adox and found co-localization of both glucagon and insulin in pancreatic islets and co-expression of glucagon and the β cell marker PDX1 [83]. It seems that a single epigenetically active molecule is able to induce cell fate conversion. Without the introduction of exogenous transcription factors, small molecules can potentially generate neural cells from somatic cells. Histone deacetylase inhibitors (VPA, NaB, and TSA) and histone demethylase inhibitor parnate coupled with other appropriate chemical drugs regulating the developmental signaling pathways can turn fibroblasts or astrocytes into neural cells or neural stem/progenitor cells [71, 84–87]. Likewise, VPA, histone methyltransferase inhibitors (DZNep, BIX-01294, and vitamin C), and the histone demethylase inhibitor AS8351 together with other chemical compounds derive cardiomyocytes from fibroblasts [68, 69, 88]. In summary, small molecules as inhibitors or activators of histone-modifying enzymes might promote transdifferentiation.

Table 4 Small molecule-mediated modulation of histone-modifying enzymes during transdifferentiation

Small molecules	Targeted enzymes	Effects on transdifferentiation	Ref
Trichostatin A (TSA)	HDACs	Promotes small molecule-mediated transdifferentiation of fibroblasts into NPCs	[84]
Valproic acid (VPA)	HDACs	Promotes small molecule-mediated transdifferentiation of fibroblasts into neurons	[86]
		Promotes small molecule-mediated transdifferentiation of astrocytes into NPCs	[85]
		Promotes small molecule-mediated transdifferentiation of fibroblasts into NPCs	[85]
		Promotes transdifferentiation of fibroblasts into cardiomyocytes	[88]
Sodium butyrate (NaB)	HDACs	Promotes small molecule-mediated transdifferentiation of fibroblasts into NPCs	[84, 87]
Parnate	H3K4 demethylases	Promotes Oct4-mediated transdifferentiation of fibroblasts into cardiomyocytes	[82]
		Promotes transdifferentiation of fibroblasts into cardiomyocytes	[88]
		Promotes small molecule-mediated transdifferentiation of fibroblasts into NPCs	[71]
Adox	Histone methyltransferases	Promotes transdifferentiation of pancreatic α cells into pancreatic β cells	[83]
DZNep	H3K9 methyltransferases	Promotes transdifferentiation of fibroblasts into cardiomyocytes	[88]
BIX-01294	H3K9 methyltransferases	Promotes small molecule-mediated transdifferentiation of fibroblasts into cardiomyocytes	[68]
Vitamin C	H3K9 methyltransferases	Promotes transdifferentiation of fibroblasts into cardiomyocytes	[69]
AS8315	H3K4 demethylases	Promotes small molecule-mediated transdifferentiation of fibroblasts into cardiomyocytes	[68]

HDACs Histone deacetylases, *NPCs* Neural progenitor cells

Conclusion

Reprogramming and transdifferentiation provide desirable strategies to obtain the wanted cells for regenerative medicine. Currently, numerous somatic cells can convert into pluripotent stem cells or another lineage type. The processes are primarily driven by epigenetic changes. Histone modifications as the major epigenetic regulator can alter chromatin status, thereby regulating the turn-on or –off of the genetic program. Some pre-existing histone marks (e.g., H3K9me3) in the somatic genome restrict the initial binding of reprogramming factors, impeding reprogramming. Moreover, some repressive histone marks inhibit other inappropriate genes (including the target cell-associated genes, e.g., pluripotency genes), and some active histone marks maintain somatic genes. Therefore, modulating histone modifications will promote the reprogramming and transdifferentiation. The post-translational modifications of histones are executed by histone-modifying enzymes. Although reprogramming factors, lineage master transcription factors, and small molecules potentially alter histone modifications, how they reset the entire chromatic landscape remains unclear. It is therefore necessary to unravel the mechanisms by which they change histone modification patterns. Accelerating the changes of histone modifications artificially has been emerging as an effective strategy to enhance cell fate conversion, for example genetic or small molecule-mediated modulation of histone-modifying enzymes. Modulating histone modifications can reinforce the regeneration power by enhancing good natural transdifferentiation or attenuate the fibrosis by preventing myofibroblast transdifferentiation. Nevertheless, genetically or chemically modulating histone modifications are global and unspecific, possibly activating or silencing inappropriate or risk genes (e.g., oncogenes or tumor suppressor genes). If the dynamic changes of certain histone mark (e.g., H3K27me3), its target genes, and its specific histone-modifying enzyme, are made clear during reprogramming and transdifferentiation, we can definitely shift the histone modification pattern towards that facilitating the target cell-specific gene program at the right time by regulating its modifying enzyme expression or activity. The single-cell analysis combined with the genome-wide analysis may help us to deeper understand the dynamic changes of histone modifications during cell fate conversion [23, 89, 90].

Most of our current knowledge about roles of histone modification in cell fate conversion stems from pluripotency reprogramming. Although some reached conclusions appear to be relevant also for transdifferentiation, it is useful to investigate the transdifferentiation model because the generation of desired cell types through transdifferentiation is more effective and safer. The transdifferentiation process does not pass through a pluripotent intermediate at gene expression level, without activating pluripotent genes. However, whether transdifferentiation passes through a less-differentiated state at the epigenetic level (e.g., a transient, open chromatin state) will need to be addressed. If it is true, we can adjust the duration and extent of the less-differentiated state to allow for generating our desired cells, such as somatic stem cells, lineage-committed progenitor cells, or specific mature cells. We hopefully improve the plasticity of somatic cells and generate new more cells easily.

Most experiments regarding histone modifications focus on histone marks and their modifiers but ignore their interaction with other epigenetic regulators such as DNA methylation, chromatin remodeling, and non-coding RNA. Many genes are simultaneously by diverse epigenetic regulators, which requires us to investigate how histone modifications work together with other epigenetic regulators to dynamically change chromatic state and gene expression.

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Compliance with Ethical Standards

Conflicts of Interest The authors indicate no potential conflicts of interest.

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