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Epigenetic Modulations of Induced Pluripotent Stem Cells: Novel Therapies and Disease Models

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Abstract

Recent breakthroughs in induced pluripotent stem cell (iPSC) technology hold promise for novel cell-based therapies as well as for effective drug development. The therapeutic potential of iPSCs makes it important to understand the reprogramming mechanisms and iPSC differentiation process. Epigenetic states that mediate exogenous stimulations on cell-intrinsic transcriptional features play a key role in iPSCs. This review focuses on epigenetic mechanisms that control iPSC pluripotency and differentiation. We discuss the potential application of epigenetic modulations in development of iPSC-based therapies and disease models.

Keywords

Induced pluripotent stem cells; embryonic stem cells; epigenetic modulation; reprogramming; disease model

Introduction

A rapidly expanding body of studies on embryonic stem cells (ESCs) has deepened our understanding of regulatory mechanisms of stem cell self-renewal and differentiation process for regenerative medicine. Reprogramming somatic cells to pluripotent stem cells by overexpression of four transcription factors was first achieved by Shinya Yamanaka and later independently by others, showing that iPSCs share similar self-renewal and differentiation potential with ESCs [1-5]. The resemblance of iPSCs to ESCs is determined by cell colony appearance, expression of pluripotency markers, epigenetic states, *in vivo* teratoma formation, *in vitro* differentiation, and chimeric mice with mouse iPSCs. The generation strategies of iPSCs originally require transgene integrations by lentiviral or retroviral constructs [1,3] or transposons [6,7]. Safer reprogramming methods have also been developed without genomic integration, including transfection of episomal plasmids [8], minicircle plasmids [9], proteins [10], synthetic modified RNA [11], microRNA [12],

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and Sendai virus [13]. Other strategies such as transcriptional factors, short-hairpin RNA (shRNA), human telomerase reverse transcriptase (hTERT), and chemical molecules have also been explored to improve the safety and efficiency of iPSC generation [5,14,15]. The iPSC technology provides a great opportunity for generating sufficient therapeutic cell sources that treat life-threatening diseases and injuries. It also has a huge potential for disease modeling that can facilitate rapid drug discovery and disease mechanism study [16]. However, the question of whether iPSCs display the same features of ESCs in terms of genome-wide transcription profile, differentiation potential, and immunogenicity has important implications for the full potential of iPSC technology [17]. Another topic of concern is that efficiency of iPSC generation is very low, depending on the reprogramming method and tissue origin of reprogramming cells. Understanding the reprogramming process that introduces epigenetic abnormalities into iPSCs will provide much needed insights into the safety and efficiency of iPSC technology, and therefore benefit its therapeutic use.

The reprogramming process from somatic cells to iPSCs occurs as pluripotency genes are re-activated and lineage-specific genes are down regulated. Epigenetic remodeling, which is mainly achieved by DNA methylation and histone modifications, plays a key role in the global transcriptional regulation during reprogramming [18,19]. DNA methylation is catalyzed by DNA methyltransferases, including Dnmt1, Dnmt3a, and Dnmt3b, and most commonly occurs in the self-complementary CG DNA sequence. In eukaryotic cells, DNA is closely associated with histones to form chromatin. The fundamental unit of chromatin is a nucleosome that consists of 146 bp DNA wrapped around a histone octamer. The N-terminal tail of core histone is subject to different post-translational modifications, including acetylation, methylation, phosphorylation, and ubiquitination. These modifications function to change the interactions of histones with DNA in nucleosome, giving rise to either more condensed, silent heterochromatin or to less condensed, active euchromatin (Fig. 1). The delineation of regional or global DNA methylation and histone modification patterns convey important epigenetic information on gene silencing or activation in specific developmental process and disease. These patterns of DNA methylation and histone modifications regulate gene expression without changing its DNA sequence and can be inherited over generations, a phenomenon called epigenetics.

Histone modifications play more varied roles in gene transcription. The best-characterized histone modifications are acetylation and methylation. Histone acetylation that is catalyzed by histone acetyltransferase (HAT) leads to active gene transcription. Histone methylation is catalyzed by histone methyltransferase (HMT). Histone 3 lysine methylations have been best characterized among different histone methylation types. This is a reversible modification, catalyzed by histone lysine demethyltransferase (KDM). Trimethylations at H3 lysine 4, lysine 36, and lysine 79 (H3K4me3, H3K36me3, H3K79me3) may result in transcription activation, while trimethylations at H3 lysine 9 and lysine 27, and H4 lysine 20 (H3K9me3, H3K27me3, H4K20me3) are involved in transcription repression. Interestingly, although dimethylation at H3 lysine 4 is also present on inactive loci, it is found predominantly at active loci of chromatin. Likewise, dimethylation at H3 lysine 9 and lysine 27 are mainly associated with transcriptional silencing [20,21] (Fig. 1). Comparisons of ESCs, iPSCs, and somatic cells showed significant differences of DNA methylation and histone modifications in pluripotent genes [1,3,4]. Unveiling the role of epigenomic states of reprogramming cells will provide deeper insights into iPSC generation and enhance the safety and efficiency of iPSCs. To these ends, it is also important to understand the roles of epigenetic marks as well as epigenetic modifiers in iPSC lineage commitment.

Reprogram somatic cells into iPSCs by epigenetic modulation

Somatic cells acquire epigenetic features of ESCs in iPSC generation. Although some of the important epigenetic events such as genome-wide DNA demethylation *in vitro* in the iPSC generation process have parallels *in vivo* in the fertilized egg and in primordial germ cells (PGCs) [22], epigenetic mechanisms during *in vitro* reprogramming may be very different from those *in vivo*, and need to be carefully studied. During iPSC generation, iPSC-specific genes are re-activated by DNA demethylation, loss of repressive mark H3K27me3, and regaining of H3K4me3 [23]. Dynamic profiles of H3K4 methylation in early reprogramming states suggest that H3K4me2 is lost in repressive somatic genes, but is enriched by the promoters and enhancers of pluripotency genes [24]. It is noted that the conversion of epigenetic states is incomplete in partially reprogramming cells, suggesting that iPSC generation is a step-wise and epigenetically controlled process (Fig. 2). Global epigenetic changes may explain the factor-mediated transcription reprogramming. Although the mechanisms are not well understood, it is believed that regulatory networks integrated by transcriptional factors and epigenetic modifiers have important roles in conversion of epigenetic states. Manipulation of epigenetic states by DNA demethylation and histone modifier inhibitors has been shown to improve the efficiency of somatic cell nuclear transfer (SCNT) [25,26]. It is reasonable to examine similar effects of epigenetic modulations in iPSC generation.

Transcriptional factors like Oct4, Sox2, Klf4, and c-Myc were originally found to induce pluripotency, whereas epigenetic modifiers contribute to efficient reprogramming (Fig. 2). It is believed that drugs targeting for epigenetic regulation may help eliminate somatic memory and epigenetic barriers for reprogramming. DNA methyltransferase inhibitor such as 5-azacytidine (AZA) benefits de-hypermethylation of the pluripotency genes in reprogramming. Specifically, Dnmt1 RNAi accelerates the reprogramming of somatic cells towards iPSCs [23]. DNA methylation was found to be a critical epigenetic barrier in iPSCs generated from hepatocytes, fibroblasts, and melanocytes. The incomplete DNA methylation that affects the epigenetic memory of somatic cells may lead to low efficiency of reprogramming. Knockdown of incompletely reprogrammed gene C9orf64 increases iPSC generation, suggesting that somatic memory genes may play a positive role in reprogramming [27]. The exact mechanisms of somatic memory genes in iPSC generation remain to be further investigated. It will also be interesting to know whether other incompletely reprogrammed genes are involved in somatic cell reprogramming.

Histone modifications such as methylations and acetylations in iPSC generation are under investigation as possible early reprogramming barriers. Drugs targeted to histone modifiers can improve reprogramming efficiency. For example, the chemical inhibitor BIX for histone methyltransferase G9a has been shown to stimulate iPSC generation from mouse embryonic fibroblasts [28]. BIX may compensate for the reprogramming functions of Sox2 and c-Myc, and reduce the requirement for overexpression of transcriptional factors in somatic cells. The specific role of G9a in reprogramming has been consistently shown in ESC fusion-induced reprogramming of adult neuronal cells [29]. Given that G9a mediates methylation of H3K9me2, an important challenge is to delineate the functions and regulatory mechanisms of H3K9me2 modified genes during reprogramming. Moreover, histone deacetylase (HDAC) inhibitors, valproic acid (VPA), suberoylanilide hydroxamic acid (SAHA), and trichostatin A (TSA) were also found to improve efficiency of iPSC generation. Compared to AZA, SAHA, and TSA, VPA has the greatest effect on reprogramming [30]. Although VPA treatment alone cannot reprogram fibroblasts into iPSCs, it does enable efficient induction of iPSCs with only two transcription factors, Oct4 and Sox2 [15]. The functional replacement of VPA for Klf4 indicates that epigenetic modifiers may share a similar regulatory network with transcription factors during

reprogramming. Butyrate, a naturally occurring short chain fatty acid, can also inhibit histone deacetylase. It has been shown that butyrate significantly improves mouse iPSC generation with Yamanaka factors (Oct4, Sox2, Klf4 and c-Myc) by increasing expression of pluripotency genes [31]. However, butyrate still has a positive effect on human iPSC generation in the absence of either Klf4 or c-Myc. Interestingly, the improvement in human iPSC generation efficiency due to butyrate is higher than VPA and TSA, two other histone deacetylases [32] (Table 1).

Drugs that specifically modulate histone modifications have limited availability. Because there are more varied modifiers involved in histone modifications compared with DNA methylation, development of novel epigenetic drugs such as chemical compounds and small RNA molecules to improve reprogramming efficiency will eventually benefit iPSC technology and disease modeling. The roles of specific genes targeted by epigenetic drugs in reprogramming also need to be explored. It has been shown that polycomb-group proteins that catalyze H3K27me3 are required for both ESC and iPSC reprogramming [33,34]. Additionally, Rcor2 that belongs to histone demethylase LSD1 complex can improve reprogramming efficiency. It will be interesting to know whether drugs that modulate the activity of polycomb-group proteins and LSD1 protein complex can also improve iPSC generation efficiency.

Comparison between ESCs and iPSCs in epigenetic reprogramming

An important issue in iPSC clinical application is whether iPSCs are identical to ESCs. Genome-wide studies showed that although transcription profiles of iPSCs and ESCs are globally similar to each other, iPSCs retain unique gene expression signature [35-38]. Aberrant expressions of imprinting genes such as H19 and IGF that are epigenetically controlled by DNA methylation were also detected in iPSCs [39]. Incomplete epigenetic reprogramming may lead to these transcriptional differences between iPSCs and ESCs, as unique epigenetic features have been found in iPSCs. DNA methylomes were independently applied to compare the epigenetic profiles between iPSCs and ESCs [40-42]. There are 1,175 differentially DNA methylated regions (CG-DMRs) that have been identified between iPSCs and ESCs. In addition, some hot spots of these CG-DMRs are consistently found in all iPSC lines, indicating that shared incomplete epigenetic reprogramming regions exist in iPSCs. Interestingly, the hot spots of CG-DMRs remain in different passages of iPSCs, and can be transmitted through iPSC differentiation. The DNA-hypermethylated sites in iPSCs are significantly associated with low levels of H3K27me3, whereas DNA-hypomethylated sites are correlated with H3K9me3 [42]. The high association of DNA methylation and histone modifications may provide valuable clinical markers to accurately identify reprogramming of iPSCs.

It has been noted that the detectable differences between iPSCs and ESCs vary among laboratories. Indeed, divergent culture environments and distinct methods of iPSC generation may produce lab-specific signatures of transcription and epigenetic marks [43]. Even the iPSC colonies in the same lab have been shown to be more heterogeneous than those in ESCs [38,44]. In addition, the issue of safe clinical translation of iPSCs has been raised. It may be necessary to standardize the operating procedures for reprogramming and characterization of iPSCs among different labs [45]. Finally, the relationship between iPSC functional signatures and their laboratory of origin remains to be explored.

Genetic variations between different ESC and iPSC lines may affect transcription and epigenetic states. Comparison of genetically matched iPSCs and ESCs will provide insight into reprogramming. In a murine system, iPSCs derived from mouse fibroblasts have subtle differences in transcript numbers compared to their genetically matched mouse ESCs. The

major difference is located on Dlk1-Dio3 gene cluster, which is a silencing locus in iPSCs. Further analysis showed that embryos derived from these iPSCs, in which the Gtl2 locus is repressive, have developmental defects. Moreover, acetylated H3 and H4 are significantly reduced at the Gtl2 locus in Gtl2 repressive iPSCs. Treatment with histone deacetylase inhibitor VPA reactivates Gtl2 gene transcription, and rescues developmental defects of the iPSC-derived embryos [46]. It is important to know whether the Gtl2 locus is consistently downregulated in human iPSCs, and in iPSCs derived from other somatic cells. Although high DNA methylation was observed in the silencing of the Dlk1-Dio2 cluster, it is also important to investigate whether the DNA methylation inhibitor AZA has the same rescue effect.

Epigenetic regulatory mechanisms in iPSC fate determination

Despite recent studies identifying the differences in transcriptional and epigenetic features between iPSCs and ESCs, little is known about the roles of epigenetic mechanisms in the iPSC differentiation process. The iPSCs have a more varied potential of lineage commitment, whereas the ESC differentiation is more consistent. Blood-derived iPSCs differentiate into more hematopoietic colonies than fibroblast-derived iPSCs, but the efficiency of iPSC hematopoietic differentiation is much lower than that of ESCs derived from SCNT [47]. Another study showed that granulocyte-derived iPSCs more efficiently differentiate into erythrocytes and macrophages than skeletal muscle precursor-derived iPSCs [48]. The observation above supports the hypothesis that the original somatic cell types of iPSCs can influence the efficiency of iPSC-specific differentiation.

Epigenetic memory from somatic cells may account for certain characteristics of iPSC differentiation (Fig. 2). It has been shown that iPSCs generated from different cell types have unique epigenetic features such as DNA methylation and histone modifications at cell type specific genes. Treatment with epigenetic modulators such as AZA and TSA on iPSCs derived from neural progenitors improves the efficiency of their hematopoietic differentiation. These epigenetic chemicals can activate the transcription level of blood cell specific genes to facilitate hematopoietic differentiation [47]. It is also noted that extended passaging of iPSCs can greatly decrease epigenetic differences between iPSCs and ESCs, making it possible to some day increase the yield of iPSCs derived from different cells of origin closer to their differentiation potential [48]. Other studies have also supported the notion that iPSCs become more similar to ESCs when continuously passaged [35]. Extended passaging may gradually reduce the epigenetic memory of somatic cells as DNA hyper-methylation at lineage specific genes decreases over time [48,49].

Interestingly, for specific lineage commitment, epigenetic memory may be beneficial (Fig. 2). It has been shown that beta cell-derived iPSCs differentiate more efficiently into insulin-producing cells compared to iPSCs generated from non-beta cells. Further analysis showed that the promoter regions of insulin and pdx1 genes maintain partially active chromatin states [50]. Incomplete reprogramming of lineage-specific genes that maintain epigenetic memory may eventually promote lineage commitment of iPSCs. The effect of epigenetic memory on preferential differentiation was also observed in human retinal-pigmented epithelial cell-derived iPSCs [51]. Although epigenetic memory at lineage-specific genes is difficult to eliminate completely during iPSC generation, it may actually provide more flexibility and efficiency for differentiation of particular cell types [52].

Promising application of epigenetic modulation in iPSC-based therapies and disease models

The clinical application of human ESCs so far has been hampered by controversies regarding the use of human embryos, and by technical issues of immune rejection of cell transplantation [53]. The breakthroughs that iPSC technology may offer, such as unlimited cell reservoirs, have brought new hopes for cell replacement therapy and patient-specific drug design. In sickle cell anemia mouse model, for example, genetically corrected iPSC-derived hematopoietic progenitors were shown to rescue the disease defects [54]. This “proof-of-concept” study used iPSCs in cell transplantation and gene therapy. In principle, this strategy could be used to cure any human genetic disease. Follow-up studies using iPSC technology for neurodegenerative disease therapy have provided additional support. For instance, neurons differentiated from iPSCs have been shown to integrate into a rat brain for the Parkinson’s disease model, and successfully improve the rat’s behavioral deficits [55]. Similar iPSC-based transplantation has also been reported for the treatment of heart disease [56]. Despite these advances, challenges remain to ensure the therapeutic effect and safety of iPSC technology. Because the iPSC generation process only induces epigenetic but not genetic reprogramming, researchers are now focusing on the epigenetic modulation of iPSC functions (Fig. 3).

The inhibitors of DNA methyltransferases, histone methyltransferases, and histone deacetylases that were originally studied in enzyme activities and cancer treatment have been applied in transcription factor-induced reprogramming [28-32]. These small chemical molecules improve reprogramming efficiency by reducing epigenetic barriers. Furthermore, some of the epigenetic chemicals, including VPA, AZA and TSA, can functionally improve development of iPSC-derived embryos [46] and promote iPSC differentiation [47]. Although the roles of H3K4me2/3 and H3K27me3 have been indicated in iPSC generation by high throughput DNA sequencing technologies [23,24], it is still not known which epigenetic drugs targeting these types of histone methylations can also promote transcription factor-induced reprogramming (Table 1). Ultimately, effective and safe iPSC therapy will depend on specific differentiation of iPSCs, functional integration of iPSC-derived lineages, and elimination of teratoma formation [16]. Diverse chemical compounds of epigenetic targets can provide drug sources to modulate iPSC functions [57,58], but further work is needed to evaluate the specificity and cytotoxicity of these epigenetic chemicals in iPSC pluripotency and differentiation. For examples, DNA methylation inhibitor AZA has toxic effects on normal cell cycle and growth due to inappropriate drug-induced DNA demethylation and gene expression [59]. Furthermore, none of the HDAC inhibitors used in epigenetic reprogramming have been developed to selectively target individual HDAC. It remains a difficult task to develop selective molecules towards individual HDAC inhibitors before their clinical trials in iPSC generation.

Finally, personalized drug design based on disease modeling is another attraction of iPSC technology. The patient-specific iPSCs provide a potentially unlimited reservoir of cell types with desirable traits to establish a rapid and reliable drug-screening platform (Fig. 3). In the past few years, many studies have successfully generated human iPSCs of disease models. They focus on genetically inherited diseases of neurological and cardiovascular systems [60-65]. More recently, iPSC disease models have been used for drug screening. Several research groups have made meaningful strides in drug treatment. For example, histone deacetylase inhibitor VPA as well as tobramycin have been shown to restore the expression level of survival motor neuron (SMN) protein in iPSCs generated from patients with spinal muscular atrophy [62]. In most cases, the cell types of reprogrammed somatic cells of patient and differentiated cells from iPSCs are not the same. Epigenetic memory from somatic cells may affect the generation of cells that accurately model the disease in question.

Another question is that most of the differentiated cells from iPSCs and ESCs resemble fetal or neonatal cells [66]. It is unclear whether these immature cells that carry disease genetic mutations can reproduce the disease features *in vitro*. Finding specific and sensitive drug targets for complete epigenetic reprogramming and iPSC differentiation is still a major challenge for researchers.

Concluding Remarks

The rapid progress in iPSC technology holds tantalizing promise for enabling cell replacement therapy and personalized drug discovery. Epigenetic research has supplied a plethora of potential drug targets to improve iPSC generation and cell fate determination. Although there is now compelling evidence that DNA methylation and histone modifications are important epigenetic mechanisms involving in reprogramming and differentiation, this field is still in its infancy, and issues concerning safety and efficacy of epigenetic modifiers in iPSC generation are unresolved. Due to our limited knowledge of epigenetic reprogramming, greater efforts need to be made to reveal functions of epigenetic marks and modifiers that have not yet been analyzed. A better understanding of epigenetic regulation in reprogramming is expected to benefit iPSC-based disease modeling and novel drug development.

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Reference

1. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126(4):663–676. [PubMed: 16904174]
2. Okita K, et al. Generation of germline-competent induced pluripotent stem cells. *Nature*. 2007; 448(7151):313–317. [PubMed: 17554338]
3. Yu J, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007; 318(5858):1917–1920. [PubMed: 18029452]
4. Wernig M, et al. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature*. 2007; 448(7151):318–324. [PubMed: 17554336]
5. Park IH, et al. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature*. 2008; 451(7175):141–146. [PubMed: 18157115]
6. Woltjen K, et al. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature*. 2009; 458(7239):766–770. [PubMed: 19252478]
7. Kaji K, et al. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature*. 2009; 458(7239):771–775. [PubMed: 19252477]
8. Yu J, et al. Human induced pluripotent stem cells free of vector and transgene sequences. *Science*. 2009; 324(5928):797–801. [PubMed: 19325077]
9. Jia F, et al. A nonviral minicircle vector for deriving human iPS cells. *Nature methods*. 2010; 7(3):197–199. [PubMed: 20139967]
10. Kim D, et al. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell*. 2009; 4(6):472–476. [PubMed: 19481515]
11. Warren L, et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell*. 2010; 7(5):618–630. [PubMed: 20888316]
12. Nava-Danso F, et al. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. *Cell Stem Cell*. 2011; 8(4):376–388. [PubMed: 21474102]
13. Ban H, et al. Efficient generation of transgene-free human induced pluripotent stem cells (iPSCs) by temperature-sensitive Sendai virus vectors. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108(34):14234–14239. [PubMed: 21821793]

14. Li H, et al. The Ink4/Arf locus is a barrier for iPS cell reprogramming. *Nature*. 2009; 460(7259): 1136–1139. [PubMed: 19668188]
15. Huangfu D, et al. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nature Biotechnology*. 2008; 26(11):1269–1275.
16. Sun N, et al. Human iPS cell-based therapy: considerations before clinical applications. *Cell Cycle*. 2010; 9(5):880–885. [PubMed: 20160515]
17. Wu SM, Hochedlinger K. Harnessing the potential of induced pluripotent stem cells for regenerative medicine. *Nature Cell Biology*. 2011; 13(5):497–505.
18. Bernstein BE, et al. The mammalian epigenome. *Cell*. 2007; 128(4):669–681. [PubMed: 17320505]
19. Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. *Nature Reviews Genetics*. 2009; 10(5):295–304.
20. Sims RJ 3rd, Reinberg D. Histone H3 Lys 4 methylation: caught in a bind? *Genes Dev*. 2006; 20(20):2779–2786. [PubMed: 17043307]
21. Huang C, et al. Dual-specificity histone demethylase KIAA1718 (KDM7A) regulates neural differentiation through FGF4. *Cell Res*. 2010; 20(2):154–165. [PubMed: 20084082]
22. Hajkova P. Epigenetic reprogramming--taking a lesson from the embryo. *Current Opinion in Cell Biology*. 2010; 22(3):342–350. [PubMed: 20537882]
23. Mikkelsen TS, et al. Dissecting direct reprogramming through integrative genomic analysis. *Nature*. 2008; 454(7200):49–55. [PubMed: 18509334]
24. Koche RP, et al. Reprogramming factor expression initiates widespread targeted chromatin remodeling. *Cell Stem Cell*. 2011; 8(1):96–105. [PubMed: 21211784]
25. Kishigami S, et al. Significant improvement of mouse cloning technique by treatment with trichostatin A after somatic nuclear transfer. *Biochemical and Biophysical Research Communications*. 2006; 340(1):183–189. [PubMed: 16356478]
26. Blelloch R, et al. Reprogramming efficiency following somatic cell nuclear transfer is influenced by the differentiation and methylation state of the donor nucleus. *Stem Cells*. 2006; 24(9):2007–2013. [PubMed: 16709876]
27. Ohi Y, et al. Incomplete DNA methylation underlies a transcriptional memory of somatic cells in human iPS cells. *Nature Cell Biology*. 2011; 13(5):541–549.
28. Shi Y, et al. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. *Cell Stem Cell*. 2008; 3(5):568–574. [PubMed: 18983970]
29. Ma DK, et al. G9a and Jhd2a regulate embryonic stem cell fusion-induced reprogramming of adult neural stem cells. *Stem Cells*. 2008; 26(8):2131–2141. [PubMed: 18535151]
30. Huangfu D, et al. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nature Biotechnology*. 2008; 26(7):795–797.
31. Liang G, et al. Butyrate promotes induced pluripotent stem cell generation. *The Journal of Biological Chemistry*. 2010; 285(33):25516–25521. [PubMed: 20554530]
32. Mali P, et al. Butyrate greatly enhances derivation of human induced pluripotent stem cells by promoting epigenetic remodeling and the expression of pluripotency-associated genes. *Stem Cells*. 2010; 28(4):713–720. [PubMed: 20201064]
33. Pereira CF, et al. ESCs require PRC2 to direct the successful reprogramming of differentiated cells toward pluripotency. *Cell Stem Cell*. 2010; 6(6):547–556. [PubMed: 20569692]
34. Zhang Z, et al. PRC2 complexes with JARID2, MTF2, and esPRC2p48 in ES cells to modulate ES cell pluripotency and somatic cell reprogramming. *Stem Cells*. 2011; 29(2):229–240. [PubMed: 21732481]
35. Chin MH, et al. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell*. 2009; 5(1):111–123. [PubMed: 19570518]
36. Marchetto MC, et al. Transcriptional signature and memory retention of human-induced pluripotent stem cells. *PloS One*. 2009; 4(9):e7076. [PubMed: 19763270]
37. Chin MH, et al. Molecular analyses of human induced pluripotent stem cells and embryonic stem cells. *Cell Stem Cell*. 2010; 7(2):263–269. [PubMed: 20682452]

38. Narsinh KH, et al. Single cell transcriptional profiling reveals heterogeneity of human induced pluripotent stem cells. *The Journal of Clinical Investigation*. 2011; 121(3):1217–1221. [PubMed: 21317531]
39. Pick M, et al. Clone- and gene-specific aberrations of parental imprinting in human induced pluripotent stem cells. *Stem Cells*. 2009; 27(11):2686–2690. [PubMed: 19711451]
40. Deng J, et al. Targeted bisulfite sequencing reveals changes in DNA methylation associated with nuclear reprogramming. *Nature Biotechnology*. 2009; 27(4):353–360.
41. Doi A, et al. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nature Genetics*. 2009; 41(12):1350–1353. [PubMed: 19881528]
42. Lister R, et al. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature*. 2011; 471(7336):68–73. [PubMed: 21289626]
43. Newman AM, Cooper JB. Lab-specific gene expression signatures in pluripotent stem cells. *Cell Stem Cell*. 2010; 7(2):258–262. [PubMed: 20682451]
44. Masaki H, et al. Heterogeneity of pluripotent marker gene expression in colonies generated in human iPS cell induction culture. *Stem Cell Research*. 2007; 1(2):105–115. [PubMed: 19383391]
45. Loh KM, Lim B. Recreating pluripotency? *Cell Stem Cell*. 2010; 7(2):137–139. [PubMed: 20682438]
46. Stadtfeld M, et al. Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. *Nature*. 2010; 465(7295):175–181. [PubMed: 20418860]
47. Kim K, et al. Epigenetic memory in induced pluripotent stem cells. *Nature*. 2010; 467(7313):285–290. [PubMed: 20644535]
48. Polo JM, et al. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nature Biotechnology*. 2010; 28(8):848–855.
49. Nishino K, et al. DNA methylation dynamics in human induced pluripotent stem cells over time. *PLoS Genetics*. 2011; 7(5):e1002085. [PubMed: 21637780]
50. Bar-Nur O, et al. Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet Beta cells. *Cell Stem Cell*. 2011; 9(1):17–23. [PubMed: 21726830]
51. Hu Q, et al. Memory in induced pluripotent stem cells: reprogrammed human retinal-pigmented epithelial cells show tendency for spontaneous redifferentiation. *Stem Cells*. 2010; 28(11):1981–1991. [PubMed: 20882530]
52. Reik W. Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature*. 2007; 447(7143):425–432. [PubMed: 17522676]
53. Swijnenburg RJ, et al. Immunosuppressive therapy mitigates immunological rejection of human embryonic stem cell xenografts. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105(35):12991–12996. [PubMed: 18728188]
54. Hanna J, et al. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science*. 2007; 318(5858):1920–1923. [PubMed: 18063756]
55. Wernig M, et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105(15):5856–5861. [PubMed: 18391196]
56. Nelson TJ, et al. Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells. *Circulation*. 2009; 120(5):408–416. [PubMed: 19620500]
57. Medina-Franco JL, Caulfield T. Advances in the computational development of DNA methyltransferase inhibitors. *Drug Discovery Today*. 2011; 16(9-10):418–425. [PubMed: 21315180]
58. Paris M, et al. Histone deacetylase inhibitors: from bench to clinic. *Journal of Medicinal Chemistry*. 2008; 51(6):1505–1529. [PubMed: 18247554]
59. Juttermann R, et al. Toxicity of 5-aza-2'-deoxycytidine to mammalian cells is mediated primarily by covalent trapping of DNA methyltransferase rather than DNA demethylation. *Proc Natl Acad Sci U S A*. 1994; 91(25):11797–11801. [PubMed: 7527544]

60. Dimos JT, et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science*. 2008; 321(5893):1218–1221. [PubMed: 18669821]
61. Soldner F, et al. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell*. 2009; 136(5):964–977. [PubMed: 19269371]
62. Ebert AD, et al. Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature*. 2009; 457(7227):277–280. [PubMed: 19098894]
63. Park IH, et al. Disease-specific induced pluripotent stem cells. *Cell*. 2008; 134(5):877–886. [PubMed: 18691744]
64. Moretti A, et al. Patient-specific induced pluripotent stem-cell models for long-QT syndrome. *The New England Journal of Medicine*. 2010; 363(15):1397–1409. [PubMed: 20660394]
65. Carvajal-Vergara X, et al. Patient-specific induced pluripotent stem-cell-derived models of LEOPARD syndrome. *Nature*. 2010; 465(7299):808–812. [PubMed: 20535210]
66. Cao F, et al. Transcriptional and functional profiling of human embryonic stem cell-derived cardiomyocytes. *PloS One*. 2008; 3(10):e3474. [PubMed: 18941512]

Figure 1. Chromatin structure and epigenetic regulation

Nucleosome that consists of 146 bp DNA wrapped around histones is the basic repeat element of chromatin. Epigenetic modifications may occur in either DNA or N-terminal tail of histone. DNA methyltransferase (Dnmt) catalyze the transfer of a methyl group to DNA. DNA methylation may lead to silent heterochromatin. There are varied kinds of histone methylation, which is dynamically catalyzed by histone methyltransferase (HMT). Histone 3 lysine methylations that have been best characterized among histone methylation types may be classified into repressive histone marks (H3K9me3, H3K27me3, and H4K20me3) and active histone marks (H3K4me3, H3K36me3, and H3K79me3). Histone acetylation that is catalyzed by histone acetyltransferase (HAT) may give rise to the formation of active euchromatin. KDM: histone lysine demethylase; HDAC: histone deacetylase

Figure 2. Regulation of reprogramming and iPSC differentiation by epigenetic mechanisms Pluripotency genes become activated throughout the reprogramming. They gradually show DNA hypomethylation and lose H3K27me3, but regain H3K4me2 and H3K4me3. Small chemical molecules that target epigenetic modifiers can improve the reprogramming efficiency. They include Dnmt1 inhibitor 5-aza-cytidine (AZA), histone methyltransferase G9a inhibitor BIX, and histone deacetylase inhibitors such as valproic acid (VPA), suberoylanilide hydroxamic acid (SAHA), and trichostatin A (TSA). Epigenetic memory is a barrier to various lineage commitments of iPSCs. However, epigenetic memory may also facilitate the differentiation from iPSCs into the specific cells that are the same type as donor somatic cells.

Figure 3. Overview of epigenetic modulation in iPSC-based transplantation and disease modeling

Epigenetic modifiers such as small chemical molecules that regulate DNA methylation and histone modifications benefit complete reprogramming and improve reprogramming efficiency of patient somatic cells. In addition, these epigenetic modifiers can reduce epigenetic barriers, and therefore enhance specificity of patient iPSC differentiation for transplantation and disease modeling.

Table 1
Epigenetic drug discovery in iPSC reprogramming

Epigenetic marks	Protein targets	Chemical drugs	Reprogramming factors	References
DNA methylation	Dnmt1	AZA	Oct4, Sox2, Klf4, c-Myc	22
DNA methylation	C9orf64	unknown	Oct4, Sox2, Klf4, c-Myc	27
Histone acetylation	HDACs	VPA, SAHA, TSA	Oct4, Sox2	15, 30
Histone acetylation	HDACs	Butyrate	Oct4, Sox2, Klf4/c-Myc	31, 32
H3K9me2	G9a	BIX	Oct4, Klf4	28
H3K27me2/3	PcG proteins	unknown	unknown	33, 34
H3K4me2/3	unknown	unknown	unknown	23, 24