Chromatin Changes in Reprogramming of Mammalian Somatic Cells

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Abstract

Somatic cell nuclear transfer (SCNT), cell fusion, and induced pluripotent stem cells (iPSCs) technologies are three strategies that allow reprogramming somatic cells into the pluripotent state; however, the efficiency is low and the mechanisms are not fully clear. In addition, there are reports that changes in chromatin play a critical role in these reprogramming strategies by modulating binding of transcription factors to their targets. In this review, we mainly discuss inactivation of the X chromosome, chromatin decondensation and remodeling, histone modifications, and histone variants in the three strategies. This review will provide an insight for future nuclear reprogramming research.

Introduction

ALL TYPES OF MAMMALIAN SOMATIC CELLS are derived from a zygote and share identical genetic information despite the significant differences in cell morphology and function. There are about 200 different somatic cell types in human body (*i.e.*, epithelial, connective, muscle, neural cells),¹ and this diversity depends on epigenetics,² which makes cells very stable *in vivo*. Cells do not normally change from one type to another.

However, somatic cells can be reprogrammed experimentally into an undifferentiated state. There are three systems of nuclear reprogramming—somatic cell nuclear transfer (SCNT),^{3,4} cell fusion,⁵ and induced pluripotent stem cells (iPSCs) technology^{6,7} (Fig. 1). They all demonstrate that the epigenetic information connected with cell identity can be erased and modified. Therefore, the mechanisms of this process have been studied separately and deeply, including DNA methylation,^{8,9} chromatin remodeling,^{10,11} histone variants and modification,¹² transcriptional components,¹³ and so on. In this review, we investigate the similarities and differences of these three systems, with a focus on one of the reprogramming mechanisms—chromatin changes.

Strategies Toward Nuclear Programming

In SCNT, also termed animal cloning, a somatic nucleus is transferred into an enucleated oocyte in which undefined components initiate new transcription of the injected nucleus.^{3,4,14–16} Another route is to fuse a somatic cell with

an embryonic stem cell (ESC) or embryonic germ cell (EGC) in such a way that the somatic cell is induced to be pluripotent.^{5,17,18} In the two systems, undefined factors in the recipient, such as transcription factors, enzymes related to histone modification and chromatin remodeling, and DNA demethylases, are all involved in the reprogramming. Yamanaka and colleagues have used defined factors (Sox2, Oct4, c-Myc, and Klf4) to reprogram both mouse and human somatic cells into iPSCs.^{6,7} Furthermore, mouse iPSCs are able to produce fertile offspring by tetraploid complementation assays (TCA), which are considered as the most strict tests for pluripotency.^{19,20} However, the efficiency of all the three strategies of nuclear reprogramming is still very low and the entire mechanism is not completely understood.

Inactivation of the X Chromosome

X chromosome inactivation (XCI) initially occurs on the paternal X chromosome in the somatic cells of mouse²¹ and results in the silencing of one X chromosome in female mammals via a dosage compensation mechanism. For the past few years, this phenomenon has been widely studied because about half of the genes responsible for resisting cellular reprogramming exist on the inactive X chromosome (Xi).²² Nolen et al. reported that the inactivated somatic X chromosome can be reactivated, but in an incomplete way, resulting in aberrant expression of X-linked genes and a mixture of cells at each stage of development.²³ Inoue et al. found that deletion of Xist, which is a noncoding RNA that inactivates one of the X chromosome

FIG. 1. Different strategies toward nuclear reprogramming. (A) Somatic nuclear transfer. The nucleus from a somatic donor cell is transferred into an enucleated oocyte. Cells from the inner cell mass (ICM) of the embryo obtained are pluripotent and able to differentiate into many types of cells independently from the tissue originating the donor nucleus. (B) Cell fusion. A somatic cell is fused with another pluripotent cell, mainly an embryonic stem cell (ESC) or an embryonic germ cell (EGC), resulting in pluripotent tetraploid hybrids. (C) Induced pluripotent stem cells (iPSCs). Over-expression of certain transcription factors, such as Oct4, Sox2, Klf4, and c-Myc can reprogram a somatic cell into ESC-like state. Color images available online at www.liebertpub.com/rej

in females, can increase cloning efficiency by 8- to 9-fold in SCNT.²⁴

Similarly, other groups found that the Xi is reactivated in ESC hybrids²⁵ and female iPSCs.²⁶ But the female mouse iPSCs (miPSCs) are different from their equivalent in humans. Female miPSCs show two active X chromosomes (XaXa) when reprogramming from mouse embryonic fibroblast cells (MEFs) or neural stem cells (NSCs), and this occurs at the later stage of reprogramming process.^{26–28} Female human (h) ESCs and hiPSCs in different derivation conditions show tremendous heterogeneity regarding to variable expression of Xist and are classified into subtypes.^{29,30} Most of hESCs and hiPSCs have only one X chromosome activated when cultured in atmospheric oxygen concentrations (20% O₂), but those stem cells with XaXa are cultivated under physiological O₂ environment (5% O₂).³¹ Recent research also supports the ideas that reactivation of Xi in hiPSCs depends on derivation conditions. For example, the use of leukemia inhibitory factor (LIF)-expressing SNL feeder cells³² and over-expression of the X-linked transcription factor ELK-1³³ are reported to promote reactivation of Xi in hiPSCs. Due to the existence of a tumorigenic phenotype in murine Xist deletion,³⁴ additional studies should focus on whether Xist-negative hiPSCs lines cause cancer when applied in the clinic.³⁵

Chromatin Decondensation and Remodeling

Chromatin is comprised of a string of nucleosomes that consists of 147bp of DNA surrounded by an octamer of canonical core histones H2A, H2B, H3, and H4. The histone H1 acts as a linker between two nucleosomes.³⁶ The condensation of chromatin is thought to be inhibitory to nuclear reprogramming because the compaction of DNA regulated by modifications of histone and DNA can prevent certain transcriptional regulators from binding to their recognition sites. Therefore, high mobility and decondensation of chromatin make for efficient reprogramming.³⁷ The average extent of chromatin decondensation can be estimated by nuclear volume, because massive nuclear swelling caused by global chromatin decondensation is one of the markers in SCNT and cell fusion experiments and commonly increases 10- to 30-fold compared to condensed nuclei.^{38–40} Likewise, sperm head swelling can also be seen during fertilization,⁴¹ indicating that chromatin decondensation is not merely a morphological event and those factors with the ability to facilitate access to DNA regulatory sites may aid nuclear reprogramming.

Chromosomal components are important for chromatin decondensation and remodeling. In SCNT, upon exposure to oocyte cytoplasm, components of donor nuclei chromatin

are replaced by the recipient oocyte. There are two kinds of replacement. In one type, the components in the oocyte displace their counterparts in somatic cells. When somatic nuclei are transferred to an enucleated *Xenopus* oocyte, it is the proteins of the high mobility group (HMG) mostly from the recipient cytoplasm that are incorporated into the nuclei rather than the somatic nuclei proteins.^{39,42} HMG proteins such as the highly mobile oocyte-specific linker histone H1foo or its counterpart B4 in the *Xenopus* oocyte are incorporated into somatic nuclei in place of the somatic H1. This is completed in few hours^{43,44} and is necessary for the decondensation of chromatin and reactivation of the pluripotency genes OCT4 and SOX2 in human and mouse cells.⁴⁵ Importantly, the same replacement happens after normal fertilization *in vivo*,⁴⁶ and our group has demonstrated that there also exists fast replacement of somatic H1 by H1foo in nuclear transfer in bovine cells.⁴⁷ It seems that these high-mobility proteins make the chromatin less compact, which also supports the point that the opening up of the chromatin structure is required for the nuclear reprogramming. Further research has shown that ectopic expression of H1foo in mouse ESCs can influence the expression of pluripotency-related genes by changing the methylation state of specific genes.⁴⁸ However, there is no evidence whether the function of H1foo or B4 is identical in the process of generating iPSCs.

Moreover, Tamada et al. found that nucleoplasmin (NPM), which is a chaperone of histones H2A and H2B, also seems necessary for the removal of somatic H1 without overt histone exchanges in nuclear reprogramming.¹¹ The addition of nucleoplasmin to the oocyte in SCNT results in similar gene expression in embryos resulting from *in vitro* fertilization (IVF),⁴⁹ suggesting its role in facilitating reprogramming of somatic nucleus. The second type of displacement is the supplement of somatic components from the oocyte, such as heterochromatin protein 1 (HP1).⁵⁰

Similarly, some chromatin remodelers in undifferentiated cells, including Chd1¹⁰ and BAF (Brg1/Brm-associated factor) complex members Baf155 and Brg1,^{51,52} can accelerate the generation of iPSCs by promoting the opening of chromatin. Brg1 is also essential for nuclear transfer.^{53,54} Indeed, it is likely that there are other undefined components in these reprogramming systems, and these need further investigation.

Histone Modifications

The modifications of histone are a principal epigenetic mechanism that occurs commonly on the core histone tails and plays a significant role in reprogramming by the regulation of chromatin configuration and gene expression.^{55,56} These post-translational modifications are methylation, acetylation, phosphorylation, and so on. Some of these modifications are relevant to opening the chromatin structure; phosphorylation of multiple histone H3 in interphase cells and acetylation of Lys-14 in histone H3 induced by NPM are correlated with chromosome decondensation.¹¹ Meanwhile, many researchers found that some factors involved in the histone modifications have an influence on these reprogramming systems. For example, inhibition of histone H3K9 methyltransferase SUV39H1, YY1, and especially the H3K79 histone methyltransferase Dot1L could facilitate reprogramming.⁵⁷ In contrast, polycomb group repressive complex 1 (PRC1) or polycomb group repressive

complex 2 (PRC2)-depleted ESCs lose the ability to reprogram human lymphocytes.⁵⁸

More importantly, recruitment of Eed-Ezh2 complex (components of PRC2) and methylation of histone H3 Lys-27(H3K27) catalyzed by Ezh2 are all involved in the onset of XCI in somatic cells.^{58–60} In contrast, the lack of H3K27 demethylase Utx fails to obtain pluripotency for somatic cells.⁶¹ In addition, vitamin C can increase the efficiency of iPSCs by partially influencing histone demethylases.^{62,63} Moreover, transcription initiation and reactivation of those pluripotent genes in somatic nucleus after nuclear transfer to oocytes require H3K4me2/3.⁶⁴ Consistent with this, the generation of iPSCs also needs Wdr5, the effector of H3K4 methylation.⁶⁵ Additionally, the DNA methyltransferase (DNMT) inhibitor 5-aza-cytidine⁶⁶ and the H3K9me2/3 methyltransferase G9a (also called as Kmd1c) inhibitor BIX-01294 can increase the efficiency of iPSCs generation greatly.^{67,68} Interestingly, G9a removal can also improve the reprogramming efficiency of cell fusion.⁶⁹ Much evidence supports the conclusion that H3K9 methylation is a barrier to the acquirement of pluripotency.^{57,70,71}

Recently, many studies about reprogramming focus on the methylcytosine hydroxylases ten-eleven translocation (TET) family. Costa and colleagues found that Nanog and Tet1/Tet2 synergistically enhance the efficiency of reprogramming, and Tet2 and polymerase-1 (Parp1) mainly compensates for the establishment of early epigenetic marks.^{72,73} And almost simultaneously, Tet1 was proven to facilitate inducing iPSCs greatly by promoting Oct4 demethylation and even replacing Oct4 and initiating nuclear reprogramming together with Sox2, Klf4, and c-Myc.⁷⁴ Piccolo et al. also demonstrated that Tet1 and Tet2 play different roles in erasing imprints when fusing somatic cells with EGCs.⁷⁵ Another candidate is Tcf3, whose deletion causes a decrease in H3K9me3 and increase in acetylated histone H3, resulting in the improvement of somatic cell reprogramming efficiency.⁷⁶

In addition, a dynamic balance between histone acetylation and deacetylation also plays an important role in reprogramming, which is regulated by histone acetyltransferase (HAT) and histone deacetylases (HDAC), respectively.⁷⁷ Pluripotent stem cells such as ESCs and ESC hybrids have hyper-acetylated histone H3 and H4 in the promoter regions of pluripotency-associated genes, whereas H4 is hypo-acetylated in differentiated cells.^{25,78} More importantly, much attempts to increase reprogramming focus on the histone deacetylation because it commonly can lead to repression of gene in differentiated cells. The use of the HDAC inhibitor valproic acid (VPA) and trichostatin A (TSA) often enhances the efficiency of both iPSCs and SCNT.^{79–82} Another HDAC inhibitor butyrate also plays a positive role in SCNT and cell fusion.^{83,84} According to the results mentioned above, a model of histone modification involved in chromatin decondensation in somatic reprogramming is summarized in Fig. 2. However the complexity of these histone modifications in somatic reprogramming is just beginning to be uncovered.

Incorporation of Histone Variants

Compared with other histone variants, macroH2A is distinct from its canonical counterpart H2A because of its additional extra-nucleosomal macrodomain.⁸⁵ Thus, the incorporation of macroH2A into chromosomes is considered

FIG. 2. A model of histone modification in somatic reprogramming. In somatic cells, pluripotent genes are silent when they are tightly packaged with methylated (Me) lysine 27, lysine 9 of histone H3 (H3K27, H3K9), and un-acetylated histone H3 and H4, along with a methylated CpG island where DNA is methylated to be 5-methylcytosine (5mC). When the somatic cells are reprogrammed by somatic cell nuclear transfer (SCNT), cell fusion, or defined transcription factors, the chromatin is comprised of a string of incompact nucleosomes with un-methylated H3K27, H3K9, and 5-hydroxymethylcytosine (5hmC) and hyperacetylated histones. In addition, methylated H3K4 is required in this process. Importantly, Tet is involved in converting 5mC into 5hmC, and its over-expression facilitates iPSCs reprogramming. The reversion of this repressive compact chromatin state in somatic cells to an open state in pluripotent cells requires different factors. The absence or inhibition of some methyltransferase or their components does help in the conversion in different reprogramming systems, such as DNA methyltransferase (DNMT), H3K9 methyltransferase G9a, SUV39H1, H3K27 methyltransferase, EZH2 (PRC2), H3K79 methyltransferase YY1, Dot1L, or H3K27 demethylases UTX. In addition, repressors of deacetylation, such as inhibitor of Hdac, including VPA, TSA, and SAHA, can also improve the reprogramming efficiency. Color images available online at www.liebertpub.com/rej

TABLE 1. FACTORS INFLUENCE NUCLEAR REPROGRAMMING

Mechanism	Factors	Strategy	References
Inactivation of X chromosome	Xist	SCNT	24
Chromatin decondensation and remodeling	H1foo, HP1, Brg1, nucleoplasmin	SCNT	11,45,52
	Brg1, Baf155 and Chd1	iPSC	10,50
Histone variant	macroH2A	SCNT	88–91
		iPSC	
	H3.3	SCNT	95
	H1.0	iPSC	96
Histone modification	SUV39H1, YY1, Dot1L	iPSC	56
Methylation	H3K27 (Ezh2, Utx)	iPSC	57,58,60
		Cell fusion	
	Vc	iPSC	61
	H3K4me2/3, BIX-01294	iPSC	66,67,68
		Cell fusion	
	Tet	Cell fusion	72,73,74
		iPSC	
	Tcf3	Cell fusion	75
		iPSC	
Acetylation	Hdac (VPA, TSA, SAHA)	SCNT	79–82
		iPSC	
	Butyrate	SCNT	83,84
		iPSC	
		Cell fusion	

SCNT, somatic cell nuclear transfer; iPSC, induced pluripotent stem cell.

to be one of the most extensive epigenetic chromatin changes and is usually implicated in embryonic development because the expression of macroH2A in ESCs increases along with cell differentiation.⁸⁶ However, once the macroH2A is depleted, the prenatally high expression of stem cell markers Nanog, Oct4, and Sox2 can be seen in these cells.⁸⁷ Moreover, macroH2A is rapidly separated from chromosomes and degraded during SCNT.⁸⁸ Similarly, a recently study of Gurdon group demonstrated that the incorporation of macroH2A is associated with resistance to reprogramming after SCNT, and its knockdown in MEFs makes for nuclear reprogramming,⁸⁹ suggesting its potential function in the recovery of pluripotency and the resistance to reprogramming.

In agreement with the function of macroH2A in SCNT, Pasque et al. found that this chromatin component also acted as an epigenetic barrier to induced pluripotency.^{90,91} It has been reported that macroH2A seems to restrict reprogramming by preventing histone acetylation^{92–94} and the incorporation of SWI/SNF remodeling complexes and other transcription factors to chromatin.¹² However, its exact mechanism in reprogramming is still not fully understood.

Additionally, the deposition of another oocyte-derived histone variant H3.3 on DNA can change the transcription pattern of somatic cells to an oocyte type after nuclear transfer.⁹⁵ The H1 variants also play a critical role in reprogramming and differentiation of human cells by the combination of their different subtypes with the regulatory regions of pluripotency factors or differentiation-related genes.⁹⁶

Conclusion and Future Perspective

An enucleated oocyte, a whole pluripotent cell, or defined factors can reprogram somatic cells to the pluripotent state by SCNT, cell fusion, or iPSCs technologies. Although the strategies are technically different, the central role of chromatin changes on the nuclear reprogramming is evident. They share some conserved mechanisms with each other. Factors involved in these routes as discussed above are shown in Table 1, and notably many of them come from oocytes or pluripotent cells. Despite the fact that these factors can increase the reprogramming efficiency to some extent, the efficiency is still low. The reprogramming was considered as a series of continuous fluctuations in which many factors act synergistically or discretely.⁹⁷ We suggest that the epigenetic changes of nuclear reprogramming may follow an early and late deterministic phase in turn. Pioneer transcription factors initiate reprogramming in the early stage of development by chromatin decondensation and remodeling. Chromatin modifications and/or histone variants incorporation and, subsequently, other pluripotent-related factors bind and activate their targets at a later stage of development. But in general, the chromosome must be decondensed completely in the first fluctuation, and the chromosome must be kept open so as to erase the memory of somatic cells consequently. Those pluripotent genes then get reactivated, and the pluripotent chromatin state is established. However, the interaction of cells or extrinsic signals cannot be neglected.

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Author Disclosure Statement

The authors have no conflict of interest to disclose.

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