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# The Effects of Genetic Alteration on Reprogramming of Fibroblasts into Induced Pluripotent Stem Cells

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## Abstract

Induced Pluripotent Stem Cells (iPSCs) can be generated from somatic cells by ectopic expression of Yamanaka factors (*Oct4*, *Sox2*, *Klf4* and *c-Myc*) or combination of other factors. Genetic alteration of fibroblasts exhibits an effect on reprogramming efficiency through multiple signaling pathways, including epigenetic modifications, metabolic shifts, Mesenchymal-To-Epithelial Transition (MET) and cell proliferation. In order to better understand the underlying mechanisms in cell fate determination, in this review we will summarize several genetic alterations involved in the regulation of reprogramming fibroblasts into iPSCs.

## Introduction

In 2006, the generation of Induced Pluripotent Stem Cells (iPSCs) from somatic cells was achieved by the overexpression of four defined transcription factors, classically *Oct4* (*O*), *Sox2* (*S*), *Klf4* (*K*) and *c-Myc* (*M*). IPSCs share many characteristics with Embryonic Stem Cells (ESCs), including the unlimited self-renewal capacity and the multi lineage differentiation potential [1]. During the past 12 years, significant progresses have been made in the inducible systems and the elucidation of molecular mechanisms during reprogramming. On the methodology, it has been improved with different delivery systems, including non-integrating viruses, small-molecule cocktails or reprogramming factors [2-4]. On the other hand, the effect of epigenetic modification, metabolic shift, the Mesenchymal-to-Epithelial Transition (MET) and cell proliferation on reprogramming have also been well studied [5-9]. The recent efforts have managed to increase their programming efficiency and safety. The novel techniques provide a platform for modeling human diseases, drug screening and regenerative medicine [10-12]. We here in briefly review and discuss several genetic alterations involved in the regulation of reprogramming fibroblasts into iPSCs.

## Ectopic Overexpression of Genes Regulate Pluripotency during Reprogramming

The classical reprogramming with 3F or 4F (*Oct4*, *Sox2*, *Klf4* and with or without *c-Myc*) is known to be an inefficient way. It is possible that somatic cell reprogramming is influenced by introducing one or more transcription factors. *Glis1* (*Glis family zinc finger1)*, greatly stimulates iPSC generation from fibroblasts when co-infected with *OSK* [13]. It's predicted that Glis1 activates multiple pro-reprogramming pathways, including c-Myc, Nanog, Lin28, Essrb, Wnt, MET, and etc. Similarly, *Zfp296* (Zinc finger protein 296) and *Zic3* (Zinc finger protein of the cerebellum 3) can also enhance the reprogramming efficiency [14,15]. *E-Ras* is specifically expressed in mouse embryonic stem cells (mESCs) and enforced expression of it promotes OSKM-mediated reprogramming. Notably, it accelerates the cell cycle through the JNK pathway and gives rise to cell proliferation [16]. *Bmi1* (B cell-specific Moloney Murin leukemia virus integration site 1) can replace *SKM* and reprogram mouse embryonic and adult fibroblasts into iPS cells in combination with *Oct4*. Bmi1 probably play roles by suppressing p16 Ink 4a and p19 Arf and upregulating sox2 and N-Myc [17]. IPSCs generated with *OSK* and *Tbx3* are better in germ-cell contribution to the gonads and germ-line transmission (Table 1). The transcription factor *Tbx3* may improve the quality of iPSCs via regulating pluripotency-associated and reprogramming factors [18]. Moreover, multiple epigenetic modification factors associated with DNA or histonemethylation or acetylation participate and facilitate cellular reprogramming of fibroblasts, such as TET1, JMJD2C, SIRT1/6 and MOF [19-23].

## Multiple Transgenic Cell Lines Influence Somatic Cell Reprogramming

Fibroblasts from several transgenic mice are also utilized in the generation of iPSCs. CHK1

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**Table-1:** Summary of genetic alterations on reprogramming fibroblasts into induced pluripotent stem cells.

| Gene | Function | Genetic alteration Type | Effect | Reprogramming Factors | Reference |
|---|---|---|---|---|---|
| Glis1 | Gli transcription factor | Ectopic expression | Positive | Glis1, O, S, K | Maekawa et al. [13] |
| Zfp296 | zinc finger protein | Ectopic expression | Positive | Zfp296, O, S, K, M | Fischedick et al. [14] |
| Zic3 | Zic transcription factor | Ectopic expression | Positive | Zic3, O, S, K | Declercq et al. [15] |
| E-Ras | activate PI3K and promote cell proliferation | Ectopic expression | Positive | E-Ras, O, S, K, M | Kwon et al. [16] |
| Tet1 | DNA demethylation and transcriptional reactivation | Ectopic expression | Positive | TET1, S, K, M | Gao et al. [19] |
| Sirt1 | NAD+-dependent protein deacetylases, block nuclear translocation of p53 | Ectopic expression | Positive | Sirt1, O, S, K, M | Lee et al. [20] |
| Sirt6 | regulator of transcription, genome stability, telomere integrity | Ectopic expression | Positive | Sirt6, O, S, K, M | Sharma et al. [22] |
| MOF | histone acetyltransferase | Ectopic expression | Positive | MOF, O, S, K, M | Mu et al. [21] |
| Tbx3 | transcription factor | Ectopic expression | Positive | Tbx3, O, S, K | Han et al. [18] |
| Bmi1 | essential for the self-renewal of stem cells | Ectopic expression | Positive | Bmi1, O | Moon et al. [17] |
| Tert | telomerasereverse transcriptase | Transgenic | Positive | O, S, K, M | Hidema et al. [25] |
| CHK1 | checkpoint kinase | Transgenic | Positive | O, S, K, (M) | Ruiz et al. [24] |
| Kdm4b | histone lysine demethylases | Transgenic | Positive | O, S, K, M | Wei et al. [26] |
| Ink4/Arf | tumor suppressor locus | Knockdown | Positive | shRNA-Ink4/Arf, O, S, K | Li et al. [29] |
| Gata4 | endodermal transcription factor | Knockdown | Positive | shRNA-Gata4, O, S, K, (M) | Serrano et al. [30] |
| AMPKα | tumor suppressor protein, induce autophagy | Knockdown | Negative | siRNA- AMPK**α**, O, S, K, M | Ma et al. [31] |
| Cbx3 | H3K9 methylation | Knockdown | Positive | siRNA-Cbx3, S, K, M | Sridharan et al. [23] |
| Lin-41 | a Ring finger-B box-Coiled coil protein | Knockdown | Negative | siRNA-LIN-41, O, S, K, let-7 inh | Worringer et al. [32] |
| p53 | tumor suppressor | Knockout | Positive | O, S, K | Hong et al. [34]; Brosh et al. [35] and Kinoshita et al. [36] |
| Dppa3 | germ-cell marker | Knockout | Negative | O, S, K | Xu et al. [38] |
| Tert | telomerasereverse transcriptase | Knockout | Negative | O, S, K, M | Kinoshita et al. [37] |
| FoxO3a | regulate the self-renewal and homeostasis of stem cell | Knockout | Negative | O, S, K, M | Wang et al. [9] |

(Checkpoint kinase 1) is linked to replication stress in the cell cycle. Chk1 transgenic mouse embryonic fibroblast harbor one additional allele of the Chk1 gene could reprogram more efficiently than wild type cells, which is due to reduction of reprogramming-induced replicationstress [24]. A Cre-loxP-mediated conditional transgenic mouse line, carrying the Tert (telomerasereverse transcriptase) expression cassette, enhances reprogramming via cooperating with c-Myc [25]. Additionally, MEF from transgenic mice for the inducible expression of Kdm4b have also reprogrammed nine-fold better into iPSCs through demethylation of H3K9/36me3 [26]. The reprogramming process of FoxO3a-null MEFs is delayed compared to the wild-type MEFs. And FoxO3a deficiency impairs the neuronal line age differentiation potential of the resulting iPS cells [27].

## Activation of Endogenous Gene in Fibroblasts Triggers Reprogramming

Generation of induced pluripotent stem cells typically requires the ectopic expression of exogenous transcription factors (Figure 1). Recently activation of endogenous Oct4 or Sox2 genes in fibroblasts by CRISPR system and combine with four small molecule compounds (Parnate, Chir99021, A83-01, and Forskolin) could trigger programming toward iPSCs [28]. Simultaneous remodeling of the Sox2 or Oct4 promoter and enhancer through precise epigenetic remodeling of endogenous loci could establish the pluripotency network. This method sheds light on how targeted chromatin remodeling triggers pluripotency induction.

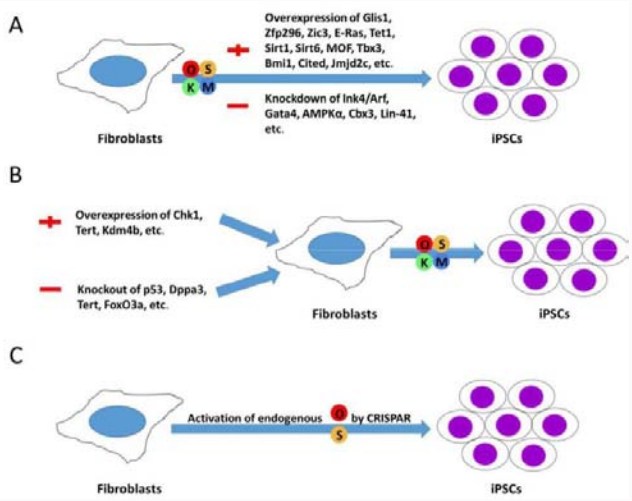


**Figure 1:** Genetic alteration of fibroblasts on reprogramming.
A) Overexpress or knockdown some genes during reprogramming.
B) Introduce or knockout some genes prior to reprogramming.
C) Activate endogenous genes by CRISPAR system to trigger reprogramming.

## The Influence of Gene Knockdown in the Generation of IPSCs

A series of specific shRNAs or siRNAs combined respectively with Yamanaka factors regulate cellular reprogramming. In 2009, Manuel Serrano et al. [29] reported that the Ink4/Arf locusblocks somatic cell reprogramming and genetic inhibition of the Ink4/Arf locus showed aprofound positive effect on the efficiency of iPSC induction [29]. Consistently, *Gata4* acts as another barrier for iPS cell reprogramming. Downregulation of endogenous *Gata4* using shRNAs during reprogramming both accelerated and increased the efficiency of the process and augmented the mRNA levels of endogenous *Nanog*, which is essential to achieve full reprogramming to naïvepluripotency [30]. On the contrary, when *AMPKα* was knocked down by specific siRNAs, reprogramming efficiency was markedly reduced [31]. And it showed that AMPK-activation-induced autophagy played a critical role in reprogramming. The let-7/LIN-41 pathway regulates reprogramming into iPSCs, and knocking down *LIN-41* with siRNAs during reprogramming with *OSK*+let-7 inhibitor also resulted in fewer iPS colonies [32].

## Fibroblasts with Gene Knockout Relate to Reprogramming Efficiency

Since the reprogramming towards iPSCs was pioneered by Takahashi and Yamanaka in 2006, lots of research groups used fibroblasts from different knockout mice in order to understand its underlying mechanisms. *p53* is known as tumor suppressor which has a pivotal involvement in cell cycle arrest, apoptosis and DNA repair [33]. Yamanaka's group revealed that they infected *p53* wild-type (*p53*+/+) mouse embryonic fibroblasts (MEF), *p53* heterozygous (*p53*+/-) mutant MEF, as well as *p53*-null (*p53*-/-) MEF with retroviruses encoding OSK and obtained about a fivefold increase of iPSC colonies from *p53*+/- MEF and dramatically more colonies from *p53*-/-MEF compared with *p53*+/+ fibroblasts [34]. Furthermore, loss of *p53* also accelerates iPSC colony formation and is less susceptible to differentiate [35,36]. Apart from *p53*, telomerase which has a function in telomere elongation plays a critical role in reprogramming and self-renewal of iPSCs. Tail-Tip Fibroblasts (TTFs) from Telomerase Reverse Transcriptase Knockout (TERT-KO) mice were tested and iPSCs were substantially reduced, which attributes tochromosomal instability [37]. Reprogramming of somatic cells to iPSCs often comes out different levels reprogrammed iPSCs, such as, partially reprogrammed iPSCs (pre-iPSCs), low-grade chimera forming iPSCs and high-grade fully reprogrammed iPSCs. Dppa3 is a germ cell marker that expressed only in low grade and high grade iPSCs. Reprogramming of *Dppa3*-knockout fibroblasts with *OSKM* generated only pre-iPSCs that failed to express endogenous Oct4 and inactivate exogenous reprogramming factors. However, this case can be rescued by Vitamin C or exogenous *Dppa3*. Exogenous *Dapp3* can enhance reprogramming and generating high-grade iPSCs. Dapp3 probably worked by antagonizing Dnmt3a to Dlk1-Dio3 locus during somatic cells reprogramming [38].

## Conclusion

In conclusion, genetic alteration of fibroblasts combined with Yamanaka factors or prior to reprogramming has an effect on re-establishment and maintenance of pluripotency via affecting signaling networks of epigenetic modifications, metabolic shifts, MET and cell cycle. Besides, utilizing transient serum starvation induces cell cycle synchronization and it promotes the METand facilitates reprogramming [39]. Recent study has further proved that serum starvation would stimulate rDNA transcription reactivation and overcome the epigenetic barrier to pluripotency [40].

IPSCs could also be induced from mouse fibroblasts by full chemicals [41-43]. The mechanisms underlying chemical reprogramming are largely elusive. Future studies were needed to clarify the roles of genetic factors and the underlying mechanisms by interacting with different small-molecule cocktails in the cell fate determination.

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