



# 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.

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## 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 iPSCs 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 germline 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 histone methylation 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

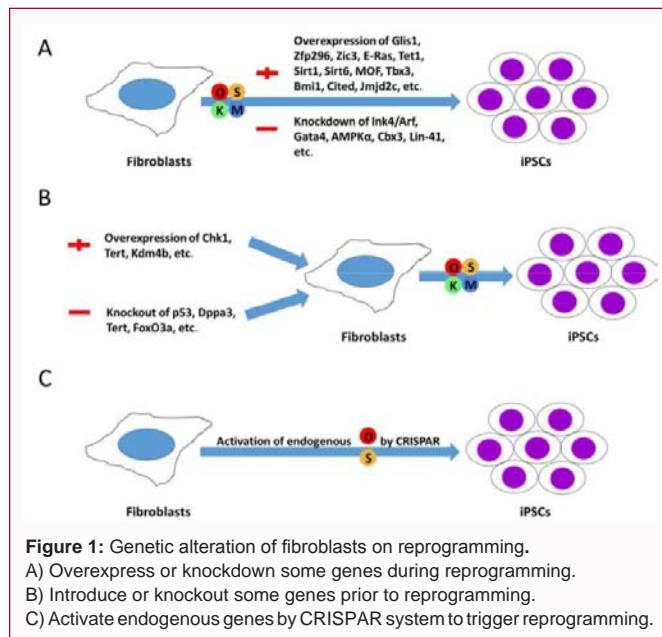
**Table-1:** Summary of genetic alterations on reprogramming fibroblasts into induced pluripotent stem cells.

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 <sup>+</sup> -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]
AMPK $\alpha$	tumor suppressor protein, induce autophagy	Knockdown	Negative	siRNA- AMPK $\alpha$ , 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 replication stress [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 iPSCs [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.



## 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* locus blocks somatic cell reprogramming and genetic inhibition of the *Ink4/Arf* locus showed a profound positive effect on the efficiency of iPSC induction [29]. Consistently, *Gata4* acts as another barrier for iPSC 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 naïve pluripotency [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 iPSC 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 to chromosomal instability [37]. Reprogramming of somatic cells to iPSCs often comes out different levels of 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 *Dppa3* can enhance reprogramming and generating high-grade iPSCs. *Dpp3* 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 MET and 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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