Cell and Tissue Engineering

AIMS Cell and Tissue Engineering, 1 (1): 31-46
DOI: 10.3934/celltissue.2017.1.31
Received: 07 April 2017
Accepted: 16 June 2017
Published: 19 June 2017

http://www.aimspress.com/journal/CTE

Review

Human Mutations Affecting Reprogramming into Induced Pluripotent Stem Cells

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Abstract: The development of induced pluripotent stem cells (iPSCs) provides unprecedented opportunities for life sciences, drug discovery, and regenerative medicine. iPSCs have been generated from somatic cells in many patients with various genetic diseases carrying specific mutations. However, the efficiency of iPSC generation is quite low. Less than 1% of human primary somatic cells can usually turn into iPSCs. Previous studies have revealed that cellular signaling pathways, epigenetic status, and cellular senescence were major barriers to iPSC generation. Serendipitously in some cases, human mutations themselves affect the reprogramming efficiency of iPSC generation as well as cellular phenotypes recapitulating their disease symptoms. Mutations, which cause altered DNA repair (*e.g.*, Ataxia-Telangiectasia, fanconi anemia and DNA Ligase IV (LIG4) syndrome), premature aging (*e.g.*, Hutchinson–Gilford progeria syndrome and Néstor–Guillermo progeria syndrome), altered telomere homeostasis (*e.g.*, dyskeratosis congenita), mitochondrial respiratory dysfunction, chromosomal abnormalities, and fibrodysplasia ossificans progressiva, have all been shown to affect the reprogramming efficiency of somatic cells to iPSCs. In this review, the effects of such mutations are summarized and the methods which have been employed to rescue efficient iPSC generation from mutant cells is discussed. Although the mutations affecting reprogramming processes are rare, these mutations have been invaluable to the elucidation of reprogramming mechanisms and to the development of improved reprogramming technologies.

Keywords: Induced pluripotent stem cells; mutations; reprogramming; ataxia-telangiectasia; fanconi anemia; dyskeratosis congenital; LIG4 syndrome; mitochondrial respiratory dysfunction; fibrodysplasia ossificans progressive; p53; chromosomal abnormality

1. Introduction

Methods, by which to generate induced pluripotent stem cells (iPSCs), were developed by transducing defined transcription factors, such as *OCT4, SOX2*, *KLF4*, and *MYC*, into somatic cells [1,2]. Such transcription factors regulate the expression of genes important for self-renewal and pluripotency. However, only a small proportion of the transduced cells are reprogrammed to generate iPSCs successfully [3], which is a major road block to applying this technology for biomedicine. Previous studies have revealed several causes of the low efficiency of iPSC generation. iPSC generation requires specific activation and/or inhibition of cell signaling pathways induced by various cytokines and/or small chemicals [4,5]. The culture conditions during iPSC generation should be optimized. Reprogramming to pluripotency involves the global changes of epigenetic modifications (e.g., gene expression patterns, DNA methylation status, and histone modifications) specific to each somatic cell type; however, the changes can be incomplete in some iPSCs, which maintain the "epigenetic memory" of original cell types [6–8]. P53- or p16-mediated cell senescence has been shown as a major barrier against successful reprogramming into iPSCs [9–13]. Furthermore, although several other genes and molecules have been shown to regulate the reprogramming process, the precise effects of each during reprogramming remain unclear.

Disease-specific iPSCs have been generated from patients with a variety of genetic diseases with either Mendelian or complex inheritance patterns and have offered an unprecedented opportunity to recapitulate both normal and pathologic human tissue formation *in vitro*, thereby enabling disease investigation and drug development [14]. Serendipitously in some cases, these mutations also affect the reprogramming processes. Such cases have been invaluable in elucidating reprogramming mechanisms and developing improved reprogramming technologies. Here, I classify those reported to date into biological mechanisms and/or pathways.

2. DNA repair

Ataxia-Telangiectasia (A-T; OMIM #208900) is a rare neurodegenerative disease characterized by early-onset progressive cerebellar ataxia, eyes and skin telangiectasia, immunodeficiency, chromosomal instability, hypersensitivity to ionizing radiation, and increased cancer risk [15]. A-T is caused by a defect in the ATM (Ataxia–telangiectasia mutated) gene [16], which produces a serine–threonine kinase activated when cells are exposed to DNA double-strand breaks (DSBs) [17]. ATM phosphorylates many proteins regulating cell cycle checkpoint, apoptotic responses, and DNA repair, including p53, Chk2, BRCA1, RPAp34, H2AX, SMC1, FANCD2, Rad17, Artemis, and Nbs1. This activity initiates

cell-cycle arrest at G_1/S, intra-S and G_2/M checkpoints and promotes DNA repair after DSBs [18]. Several groups have independently generated A-T iPSCs and demonstrated that neuronal cells differentiated therefrom were excellent models to examine A-T-associated neurodegeneration [19–23]. The reprogramming efficiency from A-T patients' somatic cells was markedly decreased compared to fibroblasts from healthy individuals [19,21,22]. Also, fibroblasts from *Atm*-deficient mice showed remarkably low efficiency of iPSC generation compared to somatic cells from wild-type mice [13,24]. These results suggested that ATM-mediated DNA-damage responses were important for reprogramming toward pluripotent state. In order to rescue the decreased iPSC generation efficiency of A-T patients' cells, Bhatt et al., expressed BCL-xL (isoform Bcl-X(L) of BCL2L1), which markedly increased the reprogramming efficiency of normal blood cells [25–27], together with reprogramming factors during iPSC generation. This method allowed the generation of A-T iPSCs with high efficiency under xeno- and feeder-free culture conditions [28]. Regarding the genomic integrity of ATM-deficient iPSCs, a study showed that they were characterized by additional copy number variations (CNVs), suggesting that ATM-mediated DNA-damage response was important for maintaining genomic integrity in iPSCs [29]; however, other studies have shown that A-T iPSCs are chromosomally stable with rates of nucleotide substitution during long-term cultures comparable to control iPSCs [21].

Dysfunctions in non-homologous-end-joining (NHEJ) repair of DNA DSBs cause several human syndromes. DNA Ligase IV (LIG4) plays an essential role in NHEJ pathway [30,31]. Mutations in LIG4 result in LIG4 syndrome (OMIM #606593), which is characterized by growth defects, microcephaly, reduced number of blood cells, increased predisposition to leukemia, and variable degrees of immunodeficiency [32–34]. The generation of LIG4 syndrome-specific iPSCs has been reported in two independent studies [35,36]. These studies demonstrated that the impairment of NEHJ-mediated-DSB repair in LIG4 iPSCs resulted in the accumulation of DSBs and enhanced apoptosis. These studies also consistently reported the decreased efficiency of iPSC generation from LIG4 syndrome patients' cells. One of the studies showed that the complementation of wild-type LIG4 partially rescued the reprogramming efficiency [36]. This study also used patients' cells with mutations in genes encoding DNA-protein kinase catalytic subunit (DNA-PKcs; OMIM #615966), and Artemis (OMIM #602450). They reported that DNA-PKcs mutant cells showed a modest decrease in reprogramming efficiency whilst Artemis mutant cells showed comparable reprograming efficiency to wild-type cells. Similarly, the reprograming efficiency of cells from individuals with mutations in Cernunos/XRCC4-LIKE FACTOR (XLF; OMIM #611291) was similar to that of healthy donors [37]. Taken together, these results indicated a critical importance of the LIG4-mediated NHEJ pathway for somatic cell reprogramming and self-renewal of iPSCs. However, there appears to be a spectrum of reprogramming defects across humans with germline mutations in NHEJ factors. Fanconi anemia (FA; OMIM #227650) is a rare recessive, autosomal or X-linked, chromosomal instability disorder, causing bone marrow failure, and increased predisposition to develop malignancies [38,39]. FA is caused by mutations in any of the 15 genes identified so far, which are involved in FA pathway. Most of FA pathway proteins form a unique nuclear protein complex that ubiquitinates FANCD2 and FANCI, leading to the formation of DNA repair reaction [40,41]. FA patients' cells displayed chromosomal

instability and hypersensitivity to the DNA crosslinking agents that have been used for the diagnosis of FA [42]. Several groups have independently generated FA-specific iPSCs from FANCA, FANCC, FANCD2, and FANCG mutant cells and demonstrated that hematopoietic lineage differentiation from FA-specific iPSCs represented a good model system to recapitulate FA-associated disease phenotypes [43–49]. All of these studies showed compromised generation or decreased efficiency of FA iPSCs and indicated that activation of FA pathway proteins was critical in the reprogramming processes. In order to rescue the generation of iPSCs from FA patients' cells, a study reported that the overexpression of human papillomavirus 16 (HPV16) E6 protein or p53 repression in combination of reprogramming factors overcame barriers to reprogramming [46]. However, the resulting FA iPSCs remained sensitive to the deficiency of the FA pathway and failed to self-renew. These results suggested that FA pathway genes are essential for DNA-repair mechanisms during reprogramming and for self-renewal of established pluripotent stem cells.

In summary, the DNA repair genes which affect the efficiency of reprogramming are shown in Figure 1. The role of these genes in reprogramming largely remains elusive but examining further the involvement of DNA repair pathways in iPSC reprogramming mechanisms would be fruitful for a more complete understanding of the process.

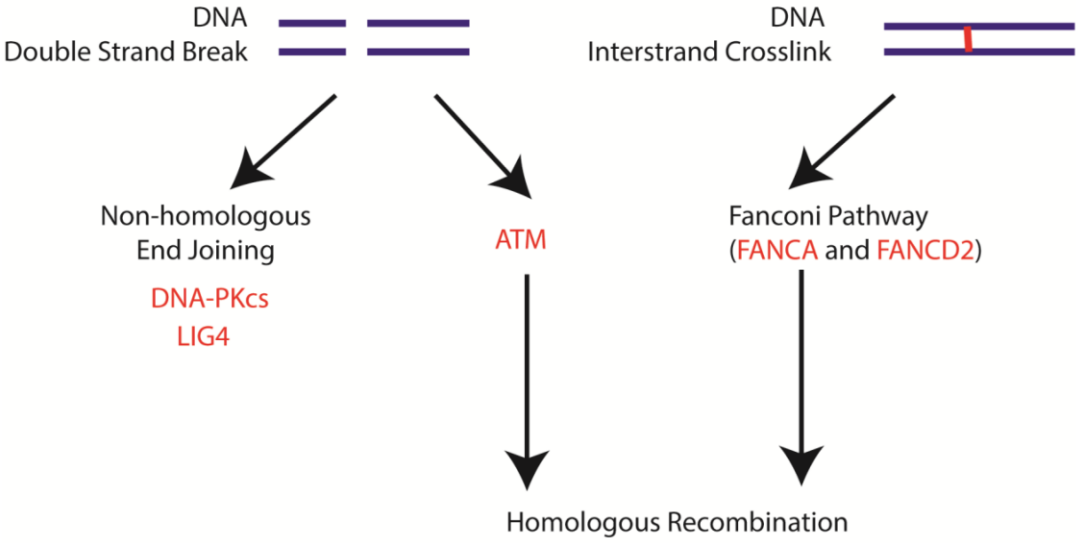

Figure 1. A figure illustrating the DNA repair genes which mutations affect reprogramming efficiency.

3. Senescence

P53-mediated cell senescence is a major barrier against successful reprogramming into iPSCs using p53-null mouse cells [9–13]. Although iPSCs from patients' cells carrying germline mutations in p53, which cause Li-Fraumeni Syndrome (LFS; OMIM #151623), have been generated using standard

Yamanaka factors, the study did not report on the efficiency of reprogramming [50]. Another study showed that the absence of p53 activity also contributed to the enhanced efficiency of iPS-like cell generation from a cancer cell line, HCT116 [51]. The generation of iPS-like cells from HCT116 cells was successful only when seven reprogramming factors were used; *OCT4*, *SOX2*, *KLF4*, *LIN28* (also known as *LIN28A*), nontransforming L-MYC, SV40 large T antigen (*SV40LT*) and small hairpin (sh) RNA against p53 [52]. These studies suggested that the inhibition of p53 was critical for the generation of iPS-like cells from cancer cells as well as from normal cells. Another study also demonstrated that both hypoxia and p53 deficiency increased iPS-like cell generation from HCT116 cells [53].

Hutchinson–Gilford progeria syndrome (HGPS; OMIM #176670) and Néstor–Guillermo progeria syndrome (NGPS; #614008) are rare premature ageing diseases. HGPS is caused by a single point mutation in the lamin A (*LMNA*) gene, resulting in the generation of progerin, a truncated splicing mutant of lamin A [54]. Progerin accumulation leads to various aging-associated nuclear defects, including disorganization of nuclear lamin A and heterochromatin loss. NGPS is caused by a homozygous mutation in *BANF1* (barrier-to-autointegration factor 1), resulting in the impairment of post-mitotic nuclear envelope assembly by the loss of direct binding to chromatin, lamin A, and emerin [55,56]. Several studies have reported the generation of iPSCs from HGPS patients' cells and have successfully demonstrated that vascular smooth muscle cells (SMCs), mesenchymal stem cells (MSCs) or fibroblasts differentiated from HGPS-iPSCs recapitulated the age-associated alterations seen in HGPS, such as premature senescence [57–60]. Two of the studies reported that no iPSC colonies were obtained from HGPS fibroblast cultures at late passages [57,58]. One of the studies also reported that the reprogramming efficiency of HGPS fibroblasts was 4-fold lower than that of parental control fibroblasts at earlier passages [57]. Another study reported that the reprogramming efficiency of NGPS fibroblasts was significantly lower than that of control fibroblasts and showed that shRNA knockdown of *BANF1* reduced the efficiency still further whilst ectopic expression of wild-type *BANF1* rescued molecular alterations and reprogramming inefficiency in NGPS fibroblasts [61]. These results indicated the causal effect of *BANF1* mutations on iPSC reprogramming efficiency and, in further experiments, demonstrated that genetic and pharmacological inhibition of NF-κB significantly increased the reprogramming efficiency of fibroblasts from NGPS patients, HGPS patients, as well as from normal aged-matched donors.

4. Telomere homeostasis

Dyskeratosis congenita (DC; OMIM #305000) is characterized by the defective maintenance of blood, pulmonary and epidermal tissues [62,63]. DC is caused by mutations in genes controlling telomere homeostasis, such as TERT, TERC, DKC1, or TINF2. Several studies have reported that there were some differences in the kinetics of telomere maintenance in iPSCs from DC patients. Agarwal *et al.* reported that *DKC1* mutant (delL37) iPSCs had elongated telomeres compared to fibroblast cells [64], but other studies have reported telomere shortening and low *TERC* expression in *TERC*, *TERT*, or *DKC1* mutant iPSCs [65–68]. Among them, two studies consistently demonstrated that the reprogramming

efficiency of DKC1 mutant cells was lower, with slower kinetics compared to normal cells [64,66]. One of the studies also showed that the hypoxic conditions (5% O_2) enabled successful reprogramming from DKC1 mutant fibroblasts [64]. These results suggested that telomere maintenance was a prerequisite for successful reprogramming into iPSCs and was associated with oxidative stress during reprogramming.

5. Chromosomal abnormalities

A study has shown that patient cells carrying 2.4 Mb duplication in chromosome 1 give rise to a lower efficiency of iPSC generation than do normal, healthy cells [69]. When we generated iPSCs from patients' cells carrying ring chromosome 13, the efficiency of iPSC generation was much higher than that from healthy control cells [70]. Taken together, these results indicate that chromosomal abnormalities affect reprogramming and the effect is dependent upon on the type of abnormality. Although it might be hard to detect which genes are involved in the reprogramming process from the patients' cells carrying chromosomal abnormalities, these cells could be useful resources for the closer examination of reprogramming mechanisms.

6. Mitochondrial respiration

Mitochondrial respiratory dysfunction caused by pathogenic mutations in mitochondrial tRNA genes emerges only when mutant mitochondrial DNA (mtDNA) proportions exceed intrinsic pathogenic thresholds. Thus, reprogramming efficiency was significantly decreased from fibroblasts with >90% mutant mtDNA (m.3243A>G), whereas fibroblasts with less mutant mtDNA showed normal reprogramming efficiency [71]. Another report has shown that mitochondrial respiratory defects caused by complex I mutations exerted only a modest decrease in reprogramming efficiency [72]. A recent study has reported a correlation between mitochondrial spare respiratory capacity in somatic cells and their reprogramming efficiency [73]. These results suggest that high variety in reprogramming efficiency among patient cells with mitochondrial respiratory dysfunction might be reflected by their different mitochondrial spare respiratory capacity. Mechanistically, we recently reported that TCL1 expression induced by KLF4 activates AKT to enhance glycolysis and inhibits mitochondrial polynucleotide phosphorylase (PnPase) to suppress oxidative phosphorylation during iPSC generation [74]. Together, these data indicate that mitochondrial respiratory activity is a key to successful reprogramming. Indeed, a low dose of resveratrol, a potent phenolic antioxidant found in grapes and red wine that also has anti-proliferative and anti-inflammatory activity, facilitated efficient cellular reprogramming from mitochondrial respiratory dysfunction patient-derived fibroblasts into iPSCs [75].

7. BMP signaling

Fibrodysplasia ossificans progressiva (FOP; OMIM #135100) is a rare congenital disease characterized by the abnormal activation of endochondral bone formation in soft tissues. Most FOP

patients carry a missense mutation (617G>A) in Activin A Type I receptor (ACVR1), leading to the hyperactivation of bone morphogenetic protein (BMP)-SMAD signaling pathway [76]. We, and other groups, have generated iPSCs from FOP patients' cells and successfully recapitulated FOP phenotypes *in vitro* [77–81]. BMP signaling has complicated roles in the maintenance of pluripotency. BMP promotes the self-renewal of mouse embryonic stem (ES) cells [82,83] whilst, in contrast, BMP inhibits the self-renewal of human pluripotent stem cells [84–88]. In mouse iPSC generation, BMP signaling facilitates their reprogramming [89]. Thus, BMP signaling has positive effects on both the induction and self-renewal of mouse pluripotent stem cells. The role of BMP signaling in human iPSC generation still remains elusive. One study has shown that fully-reprogrammed iPSCs could only be generated when BMP signaling inhibitors were employed [77], whilst we found an increased efficiency of iPSC generation from FOP patents' cells in different culture conditions and cell densities [90]. Furthermore, the efficiency of iPSC generation from normal fibroblasts was enhanced by transducing mutant ACVR1 (617G>A) or SMAD1 or by adding BMP4 protein at early periods of reprogramming. In contrast, adding BMP4 at later periods decreased iPSC generation efficiency. ID genes, transcriptional targets of BMP-SMAD signaling, are critical for iPSC generation. We subsequently found that the BMP-SMAD-ID signaling axis suppressed p16/INK4A-mediated cell senescence, a major barrier to reprogramming. However, the positive effect was easily masked by the overgrowth of non-reprogrammed cells when initial cell densities were too high, or by alteration in the periods of BMP treatment. Furthermore, the differences in the culture medium constituents may modulate the outcomes partly because recent studies have shown that Activin A, which normally activates only TGF-SMAD signaling, contributed the activation of BMP-SMAD signaling in FOP cells [91,92]. Collectively, iPSC generation from FOP patient cells are useful for clarifying the roles of cell signaling pathways in reprogramming processes.

8. Future Perspectives: rescuing methods of reprogramming in mutant cells which show decreased iPSC generation efficiency

Here, I summarize the methods used to rescue reprogramming of mutant cells which show decreased iPSC generation efficiency.

(1) Transient overexpression of the wild-type genes.

This method is simple, straightforward, and valuable to prove that the mutations themselves cause the decrease the reprogramming efficiency. However, it might also affect the outcome of disease modeling using iPSC-derived differentiated cells if the overexpressed genes remain in the reprogrammed iPSCs. For example in LIG4 syndrome iPSC generation, a study has demonstrated that the transient overexpression of wild-type LIG4 genes partially rescued the reprogramming efficiency [36].

(2) Addition of reprogramming enhancer genes.

Many genes have been reported to increase the reprogramming efficiency [93,94]. It may be helpful to use these genes to improve efficiency from mutant cells. However, the effects of these genes might be

context-dependent. For example in A-T cases, BCL-xL was used to increase the reprogramming efficiency of patient-derived blood cells [28]. It might be also useful to overexpress target genes during reprogramming, which can relieve the clinical symptoms caused by specific mutations.

(3) Treatment with specific chemicals during reprogramming.

Many chemicals have been reported to increase reprogramming efficiency [95–97]. It may be helpful to use such chemicals to improve efficiency. However, the effects of these chemicals might be context-dependent and even poorly reproducible. It might be also useful to treat with chemicals which have been shown to relieve the clinical symptoms caused by specific mutations during reprogramming. For example, a low dose of resveratrol can increase the efficient cellular reprogramming of the mitochondrial respiratory dysfunction patient-derived fibroblasts into iPSCs [75]. Furthermore, some conventional culture conditions during reprogramming might be incompatible for specific mutant cells. In these cases, culture components, such as feeder cells, growth factors, hormones, extracellular matrices, vitamins, or amino acids, could be changed to improve the reprograming efficiency [90,98–100].

(4) Hypoxic conditions.

Hypoxic conditions increase the reprogramming efficiency from normal human somatic cells [101]. This method can be generally used but the changes might be too subtle to increase effectively the reprogramming efficiency from mutant cells. For example, successful reprogramming from DC fibroblasts was achieved under low oxygen conditions (5% O_2) [64].

9. Summary

In this review, I introduce human mutations affecting reprogramming efficiency into iPSCs (summarized in Table 1). However, it remains largely unclear why and how these mutations affect the reprogramming process. I also introduce methods which have been used to rescue iPSC generation from mutant cells showing reduced or compromised reprogramming efficiency and I believe that these methods will also be useful in establishing disease-specific iPSCs from patients' cells carrying mutations involved in the reprogramming process itself.

Table 1. A summary of human gene mutations affecting reprogramming efficiency and kinetics.

Biological Type/ Pathway	*Disease Name/Type*	*Reprogramming Efficiency*
DNA repair	Ataxia-Telangiectasia (A-T; OMIM #208900)	4 % (homo) and 15 % (hetero) compared to control fibroblast (Ref. 19)
		~1 % compared to control fibroblast (Ref. 21) somewhat reduced efficiency and slower kinetics (6–9 weeks) (Ref. 22)
	LIG4 Syndrome (OMIM #606593)	0.7–17% compared to control fibroblast (Ref. 35)
		Less than 1% compared to control fibroblast (Ref. 36)

	DNA-PKcs mutation (OMEM #615966)	Slightly decrease or unchanged (Ref. 36)
	Artemis mutation (OMIM #602450)	Unchanged to control fibroblast (Ref. 36)
	Cernunos/XLF mutation (OMIM #611291)	Unchanged to control fibroblast (Ref. 37)
	Fanconi Anemia (FA; OMIM #227650)	No iPSC colonies from unmodified patients' fibroblasts (Ref. 43, 44,) Lower efficiency (~10%) to control fibroblasts (+p53 shRNA) and slower kinetics (~40 days) (Ref. 45) 0 to 0.005% to input cells (Ref. 47) No colonies with OSKM combination under hypoxic conditions, but 0.0003 to 0.008% with OSKM+LIN28 and shp53 episomal plasmids (Ref. 49)
Senescence	Hutchinson–Gilford progeria syndrome (HGPS; OMIM #176670)	No iPSC colonies were obtained at late passages (Ref. 64,65) At earlier passages, the efficiency of reprogramming of HGPS fibroblasts was 4-fold lower than parental control fibroblasts (Ref. 64).
	Néstor–Guillermo progeria syndrome (NGPS; #614008)	Threefold to eightfold reduction in the reprogramming efficiency compared to control fibroblasts (Ref. 68)
Telomere homeostasis	Dyskeratosis congenita (DC; OMIM #305000)	0 to 0.015% to input cells with slower kinetics (24–48 days) (Ref. 52) No colonies in 21% O_2 conditions, but 0.001 to 0.04% in 5% O_2 conditions (Ref. 54)
Chromosomal Abnormalities	2.4 Mb duplication in chromosome 1	Lower Eficiency (Ref. 69)
	Ring 13 chromosome	Higher Efficiency (Ref. 70)
Mitochondrial respiration	Mitochondrial respiratory dysfunction	Reprogramming efficiency was significantly decreased in patient's fibroblasts with >90% mutant mtDNA (m.3243A>G) that caused severe mitochondrial respiratory dysfunction, whereas fibroblasts with less mutant mtDNA load had normal reprogramming efficiency (Ref. 71) Only a modest decrease in reprogramming efficiency in mutant cells with mitochondrial respiratory defects caused by complex I mutations (Ref. 72). Correlation between mitochondrial spare respiratory capacity in somatic cells and their reprogramming efficiency (Ref. 73)
BMP signaling	Fibrodysplasia ossificans progressiva (FOP; OMIM #135100)	Only many differentiated colonies from FOP patients' cells with normal culture conditions; however they obtained fully-reprogrammed iPSCs only when BMP signaling inhibitors were treated (Ref. 77) In different culture conditions and cell density, an increased efficiency of iPSC generation from FOP patents' cells (Ref. 90)

Acknowledgements

YH declares that there is no conflict of interest regarding the publication of this paper.

This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (JSPS KAKENHI). The funding bodies had no role in decision to publish, or preparation of the manuscript.

Conflict of Interest

All authors declare no conflicts of interest in this paper.

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