Int J Pharm Bio Sci 2012 Oct; 3(4): (B) 845 - 850

Review Article **Cell Biology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

REVERSING THE CELL FATE: A REVIEW ON REPROGRAMMING THE CELL INTO PLURIPOTENT STAGE IN THE 21ST CENTURY

MANASH P. BARKATAKI*

Dept. of Biotechnology & Bioinformatics-Padmashree Dr. D. Y. Patil University, Navi Mumbai, India

ABSTRACT

Recent studies in the field of regenerative medicines suggesting the potential benefits of iPS cells have made the scientists to develop novel techniques which can produce iPS cells from terminally differentiated cells. The current review looks into the different factors involved in reprogramming a differentiated cell and also gives a brief overview of different approaches.

KEYWORDS: iPS cells, induced pluripotency, differentiation, reprogramming factors.

MANASH P. BARKATAKI
Dept. of Biotechnology & Bioinformatics-Padmashree Dr. D. Y. Patil University,
Navi Mumbai, India

INTRODUCTION

In sexual reproduction, when the male & female gamete fuse, they give rise to a totipotent cell (zygote) which have the unique ability of giving rise to all the cells of an organism including the extraembryonic tissues [1]. During its development, it repeatedly divides and gives more totipotent cells and its due course gets more and more specialized. Totipotent cells first give rise to the pluripotent cells which can give rise to all the cells except the extraembryonic tissue. As this process continues, pluripotent cells give rise to multipotent, then oligopotent, unipotent and finally the terminal fully differentiated cell of a system. In the past, efforts had been made to reverse the process of differentiation and thereby reprogramming fully differentiated cells to pluripotency. The approach of reprogramming a cell to its pluripotent stage is called as induced pluripotency and the cells obtained are called as induced pluripotent stem cells (iPSCs). This would help us find a solution to various diseases[1]. Studies showed that the number of cell divisions is a key parameter driving epigenetic reprogramming to pluripotency. Almost all donor cells eventually give rise to iPSCs upon continued growth and transcription factor expression the stability of which is regulated by endogenous genetic determinants and can be modified by exogenous factors [2], [3].

KEY FACTORS AND ASSOCIATED CHALLENGES

Somatic cells can be reprogrammed into induced pluripotent stem (iPS) cells by over expressing combinations of factors such as Oct4, Sox2, Klf4, and c-Myc in stoichiometric requirements [4]. Another study shows that different functional moieties of the Myc proto-oncogene products are involved in the transformation and promotion of directed reprogramming rather than one [5]. It was also found that along with the 4 main reprogramming factors, the Spalt transcription factor, Sall4 can enhance the rate of reprogramming [6]. Reprogramming is slow, stochastic and has a low frequency suggesting the existence of barriers limiting its efficiency. Studies show that senescence, DNA methylations are some of the barriers [7–9]. Suppressing the p53 pathway involved in senescence increases the no. of iPSCs formed [10]. Efficient reprogramming requires chromatin remodeling, translational regulation, RNA inhibition of transcription factors and efficient degradation of no longer needed proteins and RNAs [11–13]. Foxd3 forkhead transcription factor and mir-302 microRNA (miRNA) family (mir-302s) are found to be a key factor [14], [15]. Pol-II-based intronic miRNA expression system was used to transgenically transfect the mir-302s and was found that the cell had a highly demethylated genome [14]. G_i signaling plays a critical role in the morphology and organization of pluripotent colonies [16]. A major impediment to the use of iPS cells for therapeutic purposes has been the viral-based delivery of the reprogramming factors because multiple proviral integrations pose the danger of insertional mutagenesis [17]. The delivery of reprogrammable factors to terminally differentiated cells helped in gaining induced pluripotency in the cells either by exogenous or endogenous means. But, the reprogramming factors tend to segregate in subsequent passaging thereby losing a subset of the signature reprogramming factors. Drug treatment of the cells resulted in “secondary” iPS cell derivation only when the missing factor was introduced. This creates a defined platform for studying reprogramming mechanisms and allows screening of genetically homogenous cells for compounds that replace any transcription factor required for iPS cell derivation [18].

CURRENT SCENARIO AND PROGRESS

Several approaches had been adopted to reprogram the cell in various cells like fibroblast, adipose tissue, cardiac cells, blastocyst embryos, etc. in various organisms like mouse, rat, primate as well as in human [19–22]. An experiment carried out in 2008 used extracts of mouse embryonic stem (ES) cells and human somatic 293T cells to achieve pluripotent state [23]. Initially, only

non-terminally differentiated cells like fibroblast cells were used to produce iPSCs but in the year 2008, iPSCs were obtained from terminally differentiated B cells [24], [25]. Similarly, iPSCs were derived from patients with a variety of genetic diseases [26–28]. A year later, another paper demonstrated the use of fused mouse embryonic stem (ES) cells and human fibroblasts thereby achieving iPSCs rapidly (1 day) and efficiently (70%) [8].

REVERSING THE FATE-USING ENDOGENOUS APPROACH

Somatic cells from adult primates was reprogrammed into a pluripotent state with 3 fold increased in the pluripotent cells by using somatic cell nuclear transfer into oocytes using different nuclear donor cells [20]. Short interfering RNA (siRNA)-mediated knockdown showed that activation-induced cytidine deaminase (AID, also known as AICDA) is required for promoter demethylation and induction of OCT4 (also known as POU5F1) and NANOG gene expression [8] and thereby nuclear reprogramming. In the same year, another study showed that the cells expressing the pluripotency marker stage specific embryonic antigen 3 (SSEA3) have enhanced iPSC generation efficiency [29]. Protein induced human iPS (p-hiPS) cells were obtained from human fibroblasts by directly delivering four reprogramming proteins fused with a cell penetrating peptide (CPP) [30]. This paves the way for a safer reprogramming strategy. Studies have shown that small molecules offer an alternative to replace virally transduced transcription factors with chemical signaling cues responsible for reprogramming. In such an attempt, Klf4 was replaced by a small molecule, kenpaullone and it was observed that iPSCs were generated in lieu of Klf4 [31]. Another novel method devised in the year 2010 used low oxygen tension and a novel anti-oxidant, 4-(3,4- dihydroxy-phenyl)-derivative (DHP-d) to directly induce adipose tissue stromal cells (ATSC) to de-differentiate into more primitive stem cells [32].

REVERSING THE FATE-USING VECTOR APPROACH

A completely different approach for nuclear reprogramming was achieved by using exogenous vector means. These include use of various vectors like plasmids and viruses. iPSCs from mouse embryonic fibroblasts were obtained by transducing transcription factors expressed from doxycycline (dox) inducible lentiviral vectors and found that transgene silencing is a prerequisite for normal cell differentiation [33], [34]. This was followed by another similar research showing the use of retroviral transduction of reprogramming factors to blood cells in order to find cure for somatic mutation of hematopoietic lineages [35]. A single virus supporting efficient polycistronic expression from a single promoter for up to four reprogramming factors was used in another study [17], [36]. Months later, a single vector plasmid based reprogramming system was combined with a piggyBac transposon to achieve robust iPSCs were obtained [37], [38]. In the same year, another experiment suggested the use of episomal vectors which are non viral or which do not integrate in the host genome was found to be safer than the former integrative vectors. This study also showed the maintenance of donor cell's gene expression along with efficient generation of iPSCs [36], [39]. All the hype around the iPSCs is due to its possibility in changing the current scenario of regenerative medicine. Using lentiviral constructs, it was seen that 3-regrogrammable factor based iPS progeny generated without the c-MYC enhances production of pluripotent stem cells with innate cardiogenic potential [40], [41].

FUTURE AHEAD

Though the iPSCs have immense potential, its use for various clinical and regenerative medicinal purposes haven't gained much pace due to low frequency and inefficient generation. Various ways have been developed but none have proved their worth. Better understanding of the molecular machinery involved in the differentiation and cell division will enable the development of more efficient techniques.

REFERENCES

1. S. Mitalipov and D. Wolf, "Totipotency, pluripotency and nuclear reprogramming," *Engineering of Stem Cells*, vol. 114, pp. 185–199, Jan. 2009.
2. J. Hanna et al., "Direct cell reprogramming is a stochastic process amenable to acceleration," *Nature*, vol. 462, no. 7273, pp. 595–601, Dec. 2009.
3. J. Hanna et al., "Metastable pluripotent states in NOD-mouse-derived ESCs.," *Cell stem cell*, vol. 4, no. 6, pp. 513-24, Jun. 2009.
4. E. P. Papapetrou et al., "Stoichiometric and temporal requirements of Oct4, Sox2, Klf4, and c-Myc expression for efficient human iPSC induction and differentiation.," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 31, pp. 12759-64, Aug. 2009.
5. M. Nakagawa, N. Takizawa, and M. Narita, "Promotion of direct reprogramming by transformation-deficient Myc," *Proceedings of the*, vol. 107, no. 32, p. 14152, Jul. 2010.
6. C. C. Wong, A. Gaspar-Maia, M. Ramalho-Santos, and R. a Reijo Pera, "High-efficiency stem cell fusion-mediated assay reveals Sall4 as an enhancer of reprogramming.," *PloS one*, vol. 3, no. 4, p. e1955, Jan. 2008.
7. A. Banito et al., "Senescence impairs successful reprogramming to pluripotent stem cells," *Genes & development*, vol. 23, no. 18, p. 2134, 2009.
8. N. Bhutani, J. J. Brady, M. Damian, A. Sacco, S. Y. Corbel, and H. M. Blau, "Reprogramming towards pluripotency requires AID-dependent DNA demethylation," *Nature*, vol. 463, no. 7284, pp. 1042–1047, Mar. 2009.
9. C. R. Farthing et al., "Global mapping of DNA methylation in mouse promoters reveals epigenetic reprogramming of pluripotency genes.," *PLoS genetics*, vol. 4, no. 6, p. e1000116, Jun. 2008.
10. T. Kawamura et al., "Linking the p53 tumour suppressor pathway to somatic cell reprogramming.," *Nature*, vol. 460, no. 7259, pp. 1140-4, Aug. 2009.
11. W. N. de Vries et al., "Reprogramming and differentiation in mammals: motifs and mechanisms," in *Cold Spring Harbor symposia on quantitative biology*, 2008, vol. 73, p. 33.
12. T. S. Mikkelsen et al., "Dissecting direct reprogramming through integrative genomic analysis," *Nature*, vol. 454, no. 7200, pp. 49–55, Jul. 2008.
13. H. Tamada and N. Kikyo, "Nuclear reprogramming in mammalian somatic cell nuclear cloning.," *Cytogenetic and genome research*, vol. 105, no. 2-4, pp. 285-91, Jan. 2004.
14. S. L. Lin et al., "Mir-302 reprograms human skin cancer cells into a pluripotent ES-cell-like state," *Rna*, vol. 14, no. 10, p. 2115, Oct. 2008.
15. Y. Liu and P. A. Labosky, "Regulation of embryonic stem cell self-renewal and pluripotency by Foxd3," *Stem Cells*, vol. 26, no. 10, pp. 2475–2484, Oct. 2008.
16. K. Nakamura, N. Salomonis, K. Tomoda, S. Yamanaka, and B. R. Conklin, "Gi-coupled GPCR signaling controls the formation and organization of human pluripotent colonies.," *PloS one*, vol. 4, no. 11, p. e7780, Jan. 2009.
17. B. W. Carey et al., "Reprogramming of murine and human somatic cells using a single polycistronic vector.," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 1, pp. 157-62, Jan. 2009.
18. S. Markoulaki et al., "Transgenic mice with defined combinations of drug-inducible reprogramming factors," *Nature biotechnology*, vol. 27, no. 2, pp. 169–171, Feb. 2009.
19. Y. F. Chou et al., "The growth factor environment defines distinct pluripotent ground states in novel blastocyst-derived stem cells," *Cell*, vol. 135, no. 3, pp. 449–461, Oct. 2008.
20. M. Sparman et al., "Epigenetic reprogramming by somatic cell nuclear transfer in primates," *Stem Cells*, vol. 27, no. 6, pp. 1255–1264, Jun. 2009.

21. S. Sugii et al., “Human and mouse adipose-derived cells support feeder-independent induction of pluripotent stem cells.,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 8, pp. 3558-63, Feb. 2010.
22. N. Sun, N. Panetta, D. Gupta, and KD, “Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells,” *Proceedings of the*, vol. 106, no. 37, pp. 15720-5, Sep. 2009.
23. T. Bru, C. Clarke, M. J. McGrew, H. M. Sang, I. Wilmut, and J. J. Blow, “Rapid induction of pluripotency genes after exposure of human somatic cells to mouse ES cell extracts,” *Experimental cell research*, vol. 314, no. 14, pp. 2634–2642, Aug. 2008.
24. J. Hanna et al., “Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency.,” *Cell*, vol. 133, no. 2, pp. 250-64, Apr. 2008.
25. W. E. Lowry et al., “Generation of human induced pluripotent stem cells from dermal fibroblasts.,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 8, pp. 2883-8, Feb. 2008.
26. I. H. Park et al., “Disease-specific induced pluripotent stem cells,” *Cell*, vol. 134, no. 5, pp. 877–886, Sep. 2008.
27. Á. Raya et al., “Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells,” *Nature*, vol. 460, no. 7251, pp. 53–59, Jul. 2009.
28. F. Soldner et al., “Parkinson’s disease patient-derived induced pluripotent stem cells free of viral reprogramming factors,” *Cell*, vol. 136, no. 5, pp. 964–977, Mar. 2009.
29. J. A. Byrne, H. N. Nguyen, and R. A. Reijo Pera, “Enhanced generation of induced pluripotent stem cells from a subpopulation of human fibroblasts.,” *PloS one*, vol. 4, no. 9, p. e7118, Jan. 2009.
30. D. Kim et al., “Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins.,” *Cell stem cell*, vol. 4, no. 6, pp. 472-6, Jun. 2009.
31. C. a Lyssiotis et al., “Reprogramming of murine fibroblasts to induced pluripotent stem cells with chemical complementation of Klf4.,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 22, pp. 8912-7, Jun. 2009.
32. M. K. Jee et al., “DHP-derivative and low oxygen tension effectively induces human adipose stromal cell reprogramming.,” *PloS one*, vol. 5, no. 2, p. e9026, Jan. 2010.
33. T. Brambrink, R. Foreman, G. Welstead, C. Lengner, and M, “Sequential expression of pluripotency markers during direct reprogramming of mouse somatic cells,” *Cell Stem Cell*, vol. 2, no. 2, pp. 151-9, Feb. 2008.
34. M. Wernig et al., “A drug-inducible transgenic system for direct reprogramming of multiple somatic cell types,” *Nature biotechnology*, vol. 26, no. 8, pp. 916–924, Aug. 2008.
35. Y. H. Loh et al., “Generation of induced pluripotent stem cells from human blood,” *Blood*, vol. 113, no. 22, p. 5476, May 2009.
36. L. Shao et al., “Generation of iPS cells using defined factors linked via the self-cleaving 2A sequences in a single open reading frame,” *Cell research*, vol. 19, no. 3, pp. 296–306, Mar. 2009.
37. K. Yusa, R. Rad, J. Takeda, and A. Bradley, “Generation of transgene-free induced pluripotent mouse stem cells by the piggyBac transposon,” *Nature methods*, vol. 6, no. 5, pp. 363–369, May 2009.
38. K. Kaji, K. Norrby, A. Paca, M. Mileikovsky, P. Mohseni, and K. Woltjen, “Virus-free induction of pluripotency and subsequent excision of reprogramming factors,” *Nature*, vol. 458, no. 7239, pp. 771–775, Apr. 2009.
39. M. C. N. Marchetto, G. W. Yeo, O. Kainohana, M. Marsala, F. H. Gage, and A. R. Muotri, “Transcriptional signature and memory retention of human-induced pluripotent stem cells.,” *PloS one*, vol. 4, no. 9, p. e7076, Jan. 2009.

40. A. Martinez-Fernandez, T. J. Nelson, Y. Ikeda, and A. Terzic, “c-MYC-Independent Nuclear Reprogramming Favors Cardiogenic Potential of Induced Pluripotent Stem Cells,” *Journal of cardiovascular translational research*, vol. 3, no. 1, pp. 13–23, Feb. 2010.

41. T. J. Nelson, A. Martinez-Fernandez, S. Yamada, C. Perez-Terzic, Y. Ikeda, and A. Terzic, “Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells,” *Circulation*, vol. 120, no. 5, p. 408, Aug. 2009.