Annals of Stem Cell Research & Therapy

Editorial
Published: 30 Jan, 2018

iPS Cell Generation: Current and Future Challenges

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Editorial

The ground breaking discovery of reprogramming somatic cells to generate induced Pluripotent Stem (iPS) cells more than a decade ago has revolutionized stem cell research attracting immense global attention [1]. These cells over come ethical issues and immune barriers linked to embryonic stem cells and restricted differentiation potential associated with adult stem cells. Importantly, these cells can be an excellent model system for disease modeling, drug testing and discovery and understanding human developmental biology. Prospectively on the success of ongoing clinical trials, these cells can be widely used for patient-specific regenerative therapy due to their remarkable ability to differentiate into any desired cell type of an adult human body.

Despite these exceptional characteristics, the generation of iPS cells faces a myriad of challenges. Here, we highlight some of the major challenges:

- Choice of the somatic cell source
- Selection of a reprogramming approach
- Low efficiency
- Slow kinetics
- Identification of "bona fide" iPS clone(s)/cells

The dilemma a researcher experiences is the selection of an ideal somatic cell source for iPS generation. Till date, a variety of cell types from different origins (Table 1) have been reported to generate iPS cells, each having its own set of advantages as well as disadvantages [2]. The primary goal is to minimize the invasiveness in deriving the somatic cells from patients for cell reprogramming. Fibroblasts are the most common cell source used for iPS generation due to its cheap and easy handling. However, isolating this somatic cell type from a patient involves an invasive procedure performing a skin punch biopsy. In addition, involvement of Mesenchymal-to-Epithelial Transition (MET) during reprogramming and retention of somatic memory of fibroblast-specific genes are the other hurdles. Absence of MET in case of other interesting cell sources like keratinocytes or urine derived renal epithelial cells due to their epithelial origin is an additional advantage. Importantly, these somatic cell sources are derived using non-invasive approaches and are easy to reprogram with higher reprogramming efficiencies compared to fibroblasts. The limiting factor of these promising cell sources is that they undergo senescence in culture after very few passages. The model choice of a somatic cell source would be to circumvent invasiveness, any chance of acquiring somatic mutations and inadvertent retention of aberrant residual epigenetic memory during reprogramming to obtain quality iPS cells for regenerative therapy. The other major challenge is the selection of an appropriate reprogramming approach to derive clinical grade iPS cells. Numerous approaches have been employed till date to generate iPS cells (Table 2) [3-20]. Integrating approaches using viral vectors with different combinations of transcription factors have been successfully employed to generate iPS cells. Although viral approaches are robust and efficient, they carry enormous risk of insertion mutagenesis and tumor formation, nullifying the clinical applicability of these cells. To overcome this safety concern, significant progress has been made in establishing non-integrating viral approaches (adenovirus, sendai virus) and non-viral approaches (plasmid transfection, piggybactransposon, mini circle vector, episomal, modified mRNA, micro RNAs, recombinant proteins and small molecules) to derive iPS cells. These approaches diminish or eliminate the possibility of any genomic alteration. However, they are labor intensive and/or are reported to be less efficient with slow kinetics and result in large number of partially reprogrammed colonies. Extensive characterization of the iPS clones/cells generated using these non-integrative approaches is essential to safeguard from adverse effects before cell therapy applications. Low efficiency and slow kinetics are other key barriers encountered by researchers

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Received Date: *10 Jan 2018*
Accepted Date: *24 Jan 2018*
Published Date: *30 Jan 2018*

Citation:
Saha B, Borgohain MP, Dey C, Thummer RP. iPS Cell Generation: Current and Future Challenges. Ann Stem Cell Res Ther. 2018; 1(2): 1007.

Table 1: Different Somatic Cell Sources for iPSC Generation.

Source Type Cell	Origin	Invasiveness
Fibroblasts	Mesodermal	Invasive
Renal epithelial cells	Mesodermal	Non-invasive (from urine)
Umbilical vein endothelial cells	Mesodermal	Non-invasive
Cord blood-derived Endothelial cells	Mesodermal	Non-invasive
Third molar mesenchymal stromal cell	Mesodermal	Non-invasive (derived from clinical waste)
Peripheral blood mononuclear cells	Mesodermal	Invasive
B lymphocytes	Mesodermal	Invasive
Hematopoietic stem cells (HSCs)	Mesodermal	Invasive
Keratinocytes	Ectodermal	Non-invasive
Melanocytes	Ectodermal	Invasive
Dental pulp stem cell	Ectodermal	Invasive
Astrocytes	Ectodermal	Invasive
Neural Stem Cells (NSCs)	Ectodermal	Invasive
Hepatocytes	Endodermal	Invasive
Pancreatic β cells	Endodermal	Invasive

Table 2: Integrative and Non-integrative approaches for iPSC Generation.

Gene Delivery Method	TF cocktail	References
Integrative Viral Approaches		
Retrovirus	OSKM	[3]
Polycistronic retrovirus	OSKM	[4]
Lentivirus	OSLN	[5]
Inducible lentivirus	OSKM/OSKMN	[6]
Inducible polycistronic lentivirus	OSKM	[7]
Non-integrative Viral Approaches		
Adenovirus	OSKM	[8]
Sendai virus	OSKM	[9]
Integrative Non-Viral Approaches		
Piggy Bac transposon based plasmid (Excisable)	OSKM	[10]
Non-integrative Non-Viral Approaches		
Plasmid transfection	OSKM	[11]
Episomal	OSNLMK+SV40LT	[12]
Mini-circle DNA vector	OSLN	[13]
Proteins	OSKM	[14]
mRNA	OSLN,OSKM/OSKML	[15,16]
microRNA	None	[17,18]
Small molecules	None	[19]

Abbreviations: TF: Transcription Factor; O: Octamer-binding Transcription Factor 4 (OCT4); S: SRY (sex determining region Y)-box 2 (SOX2); K: Kruppel-Like Factor 4 (KLF4); M: c-MYC; N, NANOG; L, Lin-28 homolog A (LIN28); SV40LT – Simian Virus 40 Large T-antigen.

during cell reprogramming. The efficiency of reprogramming is in the range of 0.0001% to 5% and the entire reprogramming process takes around ~2 to 8 weeks [21,22]. This partly depends on the choice of somatic cell source and reprogramming approach used to produce iPS cells. Various reprogramming barriers (Figure 1) like MET, senescence machinery, kinases, epigenetic roadblocks, signaling pathways such as TGF-β and Hippo, ubiquitin proteasome system, genomic instability, non-optimal stoichiometry and/or expression of reprogramming transcription factors, certain pluripotency-inhibiting micro RNAs and transcription factors, etc. that a somatic cell has to overcome to attain a pluripotent state [23]. The journey of a somatic cell to successfully reach a pluripotent state overcoming all these potent barriers naturally yields a small number of "true" or "bona fide" iPS cells, resulting in low efficiency and slow kinetics. The identification of "bona fide" iPS cells from a reprogramming dish is laborious, time consuming, expensive and requires extensive characterization of the picked clones to assess their pluripotency. Researchers devote a considerable amount of time in analyzing partially reprogrammed or pre-iPS cells in the quest for "bona fide" iPS cells, since the former are hard to distinguish morphologically from the latter. The seminal study in 2006 used Fbx15 expression for selection of iPS cells, however, these cells did not generate adult

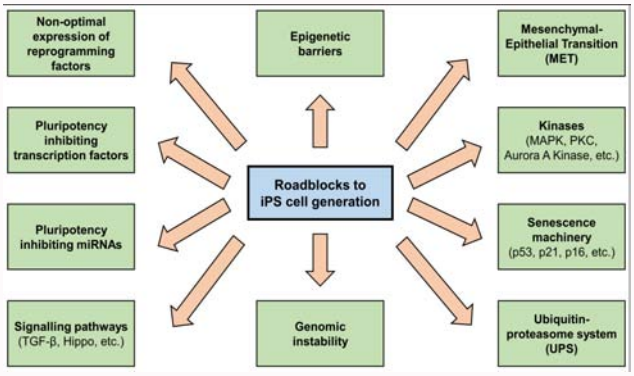

Figure 1: Different roadblocks to generation of iPS cells. (Abbreviations: miRNA, micro RNAs; TGF-β, Transforming growth factor – β; MAPK, mitogen-activated protein kinase; PKC, protein kinase C).

chimeras, and therefore were not "bona fide" iPS cells, instead were incompletely reprogrammed colonies [1]. The subsequent studies reported adult chimera competent "bona fide" iPS cells through over expression of the same cocktail of transcription factors employing selection for Oct4 and Nanog rather than Fbx15 [24,25]. Two other studies used Rex-1- [26] and UTF1-based [27,28] reporter systems to identify "bona fide" iPS cells. Using live cell imaging, Chan et al. [29] demonstrated expression of REX-1, DNMT3B and TRA-1-60 as good markers along with pro viral silencing to identify the fully reprogrammed "bona fide" iPS colonies. Recently, expression of cell surface markers SSEA-4 in the early stage and TRA-1-60 in the intermediate stage followed by silencing of retroviral genes during the late phase facilitated the easy identification of "bona fide" human iPS cells [30]. Further comprehensive studies are required to identify various markers which can be used to determine "bona fide" iPS cells with certainty.

Conclusion

iPS cells are a valuable patient-specific cell source for better understanding of basic biology, disease modeling, drug development and toxicity screening and personalized regenerative medicine. The search for a promising non-integrative reprogramming approach to generate these promising cells with high efficiency, fast kinetics and giving rise to "bona fide" genetically stable iPS cells, which successfully clears the most stringent assay for pluripotency, is what scientists are aiming for. Through this combined effort, iPS cells bring lots of hope for clinical applications for treatment and cure of a variety of debilitating diseases.

Acknowledgment

We thank all the members of Laboratory for Stem Cell Engineering and Regenerative Medicine (SCERM) for their excellent support. This work was supported by grants North Eastern Region – Biotechnology Programme Management Cell (NERBPMC), Department of Biotechnology, Government of India (BT/PR16655/NER/95/132/2015) and by IIT Guwahati Institutional Start-Up Grant.

References

1. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126(4):663-76.
2. Raab S, Klingenstein M, Liebau S, Linta L. A Comparative View on Human Somatic Cell Sources for iPSC Generation. Stem Cells Int. 2014;2014:768391.
3. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131(5):861-72.
4. Zhang Z, Gao Y, Gordon A, Wang ZZ, Qian Z, Wu WS. Efficient generation of fully reprogrammed human iPS cells via polycistronic retroviral vector and a new cocktail of chemical compounds. PloS one. 2011;6(10):e26592.
5. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007;318(5858):1917-20.
6. Maherali N, Ahfeldt T, Rigamonti A, Utikal J, Cowan C, Hochedlinger K. A high-efficiency system for the generation and study of human induced pluripotent stem cells. Cell Stem Cell. 2008;3(3):340-5.
7. Carey BW, Markoulaki S, Hanna J, Saha K, Gao Q, Mitalipova M, et al. Reprogramming of murine and human somatic cells using a single polycistronic vector. Proc Natl Acad Sci USA. 2009;106(1):157-62.
8. Zhou W, Freed CR. Adenoviral gene delivery can reprogram human fibroblasts to induced pluripotent stem cells. Stem Cells. 2009;27(11):2667-74.
9. Fusaki N, Ban H, Nishiyama A, Saeki K, Hasegawa M. Efficient induction of trans gene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proc Jpn Acad Ser B Phys Biol Sci. 2009;85(8):348-62.
10. Woltjen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, Hämäläinen R, et al. Piggybac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature. 2009; 458:766-70.
11. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. Science. 2008;322(5903):949-53.
12. Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II, et al. Human induced pluripotent stem cells free of vector and transgene sequences. Science. 2009;324(5928):797-801.
13. Jia F, Wilson KD, Sun N, Gupta DM, Huang M, Li Z, et al. A nonviral minicircle vector for deriving human iPS cells. Nat Methods. 2010;7(3):197-9.
14. Kim D, Kim CH, Moon JI, Chung YG, Chang MY, Han BS, et al. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell. 2009;4(6):472-6.
15. Yakubov E, Rechavi G, Rozenblatt S, Givol D. Reprogramming of human fibroblasts to pluripotent stem cells using mRNA of four transcription factors. Biochem Biophys Res Commun. 2010;394(1):189-93.
16. Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell. 2010;7(5):618-30.
17. Anokye-Danso F, Trivedi CM, Juhr D, Gupta M, Cui Z, Tian Y, et al. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell. 2011;8(4):376-88.
18. Miyoshi N, Ishii H, Nagano H, Haraguchi N, Dewi DL, Kano Y, et al. Reprogramming of mouse and human cells to pluripotency using mature microRNAs. Cell Stem Cell. 2011;8(6):633-8.
19. Hou P, Li Y, Zhang X, Liu C, Guan J, Li H, et al. Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. Science. 2013;341(6146):651-4.
20. Bellin M, Marchetto MC, Gage FH, Mummery CL. Induced pluripotent stem cells: the new patient? Nat Rev Mol Cell Biol. 2012;13(11):713-26.
21. Okita K, Yamanaka S. Induced pluripotent stem cells: opportunities and challenges. Philos Trans R Soc Lond B Biol Sci. 2011;366(1575):2198-207.

22. Singh VK, Kalsan M, Kumar N, Saini A, Chandra R. Induced pluripotent stem cells: applications in regenerative medicine, disease modeling, and drug discovery. Front Cell Dev Biol. 2015;3(2):1-18.

23. Ebrahimi B. Reprogramming barriers and enhancers: strategies to enhance the efficiency and kinetics of induced pluripotency. Cell Regen (Lond). 2015;4:10.

24. Brambrink T, Foreman R, Welstead GG, Lengner CJ, Wernig M, Suh H, et al. Sequential expression of pluripotency markers during direct reprogramming of mouse somatic cells. Cell Stem Cell. 2008;2(2):151-9.

25. Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, et al. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature. 2007;448(7151):318-24.

26. Zhong JF, Weiner L, Jin Y, Lu W, Taylor CR. A real-time pluripotency reporter for human stem cells. Stem Cells Dev. 2010;19(1):47-52.

27. Pfannkuche K, Fatima A, Gupta MK, Dieterich R, Hescheler J. Initial colony morphology-based selection for iPS cells derived from adult fibroblasts is substantially improved by temporary UTF1-based selection. PloS one. 2010;5(3):e9580.

28. Morshedi A, Soroush Noghabi M, Dröge P. Use of UTF1 genetic control elements as iPSC reporter. Stem Cell Rev. 2013;9(4):523-30.

29. Chan EM, Ratanasirintrawoot S, Park IH, Manos PD, Loh YH, Huo H, et al. Live cell imaging distinguishes bona fide human iPS cells from partially reprogrammed cells. Nat Biotechnol. 2009;27(11):1033-7.

30. Bharathan SP, Manian KV, Aalam SM, Palani D, Deshpande PA, Pratheesh MD, et al. Systematic evaluation of markers used for the identification of human induced pluripotent stem cells. Biology Open. 2017;6(1):100-8.