Induced Pluripotent Stem-cell Lines in the Clinic - Still a Long Road Ahead

Abstract

Discovery of induced Pluripotent stem cells (iPSCs) has revolutionized the fields of stem cell biology and regenerative medicine. iPSCs can now be generated through reprogramming of somatic cells from various tissues and mammalian species by ectopic expression of defined factors (Oct3/4, Sox2, c-Myc, Klf4). However, these methods of gene transduction, often involving viral vectors for delivery and genomic integration, may not be safe for clinical applications owing to high rates of malignant transformation and tumorigenesis. In this article we provide a comprehensive overview of various new strategies including but not limited to mRNA or protein based delivery and/or chemical induction of pluripotency that circumvent the use of viral vectors for gene delivery. These methodological advancements increase the ease and efficiency of reprogramming, avoiding genomic modification, thereby increasing the suitability for clinical and translational applications in humans.

Review

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Shweta Bhatt[1,2,3]* and Gongxiong Wu[1,2]

[1]*Section of Islet Cell Biology and Regenerative Medicine, Harvard Medical School, USA*
[2]*Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, USA*
[3]*Daksh Med Ventures Inc., India*

***Corresponding author:** Shweta Bhatt, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, USA, Tel: +1-415-615-2293; Email: shwetabhatt18@gmail.com

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Introduction

The seminal work by Takahashi and Yamanaka in 2006 laid the foundation for what we today know as the induced pluripotent stem cell (iPSC) field[1,2].Through an elegant set of experiments they were the first to reveal that mature somatic cells can be forced to reprogram into an embryonic-like state, with a capacity for unrestrained growth. This reprogramming and turning back the clock required expression of four essential genes, namely, *Oct4, Sox2, c-Myc and Klf4*. Several studies have drawn and demonstrated a parallel between iPSCs and embryonic stem cells (ESCs) in terms of their pluripotent potential and hence therapeutic value [3-9]. In fact, iPSCs may actually be preferable to ESCs in some therapeutic context as they do not carry a risk of immune rejection due to their patient specific nature, and are not affected by the same ethical concerns as ESCs [10]. But the methods of gene delivery to induce pluripotency while generating iPSCs have often come under scrutiny owing to the concerns on genomic alterations that some methods involve that renders them unsuitable for clinical applications in humans [11-35]. While Yamanaka et al. [1]. used the genome integrating viral vectors for gene delivery, the field has since seen an explosion of gene delivery methods including non-viral vectors, RNAs, proteins as well as reprogramming using small molecules and/or chemical treatment, all of which circumvent issues of genomic integration.

Genetic and integrating methods of reprogramming

Table1: Non-integrating methods of reprogramming to generate iPSCs.

Reprogramming Method	Species	Reference
Plasmid	Mouse and Human	Okita K et al. 2008
Adenovirus vector	Mouse and Human	Stadtfeld M et al. 2008
Excisable poly cistronic lentiviral vector	Human	Chang CW et al. 2009; Sommer C et al. 2009
OriP/EBNA1-based episomal vector	Human	Yu J et al. 2009
PiggyBac transposition	Mouse and human	Woltjen K et al. 2009; Yusa K et al. 2009
Recombinant Proteins	Mouse and human	Zhou H et al. 2009 Kim D et al. 2009
Sendai virus	Human	Fusaki N et al. 2009; Lieu PT et al. 2013
mRNA and micro RNA	Mouse and human	Judson RL et al. 2009; Mallanna SK et al. 2010; Warren L et al. 2010; Anokye-Danso F et al. 2011; Miyoshi N et al. 2011; Yoshioka N et al. 2013; Liao B et al. 2011; Subramanyam D et al. 2011
Magnetic Nano particles	Mouse and human	Lee CH et al. 2011
Small Molecules	Mouse	Pasha Z et al. 2011; Hou PP et al. 2013
Sleeping beauty transposon	Mouse and human	Davis RP et al. 2013

Pioneering work in the iPSC field suggested the requirement for stable expression of reprogramming factors like Oct4, Sox2, c-Myc, Klf4, Lin28 to induce a state of pluripotency [36-46]. This required genomic integration of these genes into the host genome using lentivirus or retrovirus vectors. While after the iPSC generation, the stable and high expression level of integrated exogenous genes gets down-regulated, yet, the possibility of tumorigenesis due to expression of oncogenes and inactivation of tumor suppressor genes cannot be ruled out. To circumvent the clinical drawbacks of genetic induction of reprogramming, owing to genomic integration, several methods have been reported for generating iPSCs through the use of non-integrating plasmid and adenovirus vectors (Table1). However, in such cases, poor reprogramming efficiency remains to be a challenge. Therefore, there is an imminent need for the establishment of new methods of reprogramming that can bypass genomic integration without compromising efficiency of reprogramming, thereby facilitating the use of iPSCs for therapeutic applications.

Non-integrating methods of reprogramming

To circumvent the clinical drawbacks of genetic induction of reprogramming, owing to genomic integration, several avenues of research have proposed alternate strategies that work with varied efficiency (Figure1). A comprehensive review and comparison of the various alternatives available is presented below.

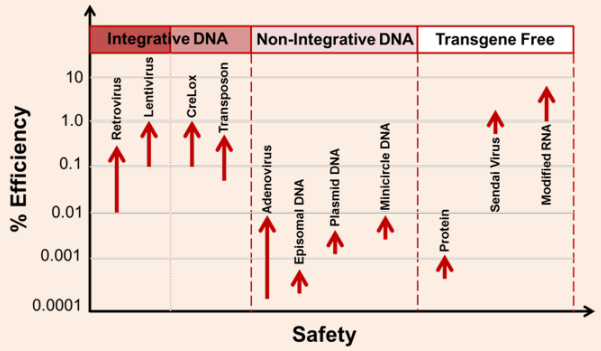

Figure1: The Evolution of Reprogramming Technologies.

Schematic representation of the contrast in the efficiency and safety of reprogramming between various technologies reported till date. (Illustration adapted from Cardiovasc. Trans. Res. 2013, 6, 956-68)

Viral vectors for generating Transgene-free iPSCs

The pioneering work for the derivation of iPSCs by Yamanaka and colleagues undertook integrative viral vectors for the delivery of reprogramming factors largely because of their obvious merits,

1) Delivery to a wide range of cell types

2) Stable and high levels of expression.

One equally obvious drawback of this methodology is the genomic integration of these exogenous gene sequences into the host genome that may lead to

1) Mutations in endogenous genes due to random integration

2) Inactivation of tumor suppressor genes

3) Hyper activation of oncogenes leading to cancer and tumorigenesis.

To circumvent these clinical drawbacks, several technical advancements have been suggested through research conducted in different parts of the world [47,48]. Soldner et al. used dox-inducible lentiviral vectors that involved the use of cre-excisable lentiviruses that upon integration can subsequently be excised from the host genome [49]. The technology was later improvised by Sommer et al. generated a novel version of this single polycistronic vector containing a reporter fluorochrome to allow direct visualization of vector excision in live iPSCs in real-time [50].

While this approach equals retroviral transduction in terms of efficiency but has the drawback of the need for additional genetic manipulations for the excision of cre-lox cassette. Another concern is the possibility of introducing chromosomal translocations during cre-mediated recombination. Lastly, a small piece of the vector is left in the integration site even after excision, which again leaves the possibility of insertional mutagenesis. To further improvise, Papapetrou et al. developed a strategy that includes additional steps for mapping the integration site in the genome to allow for the selection of clones with a single appropriate integration site for the residual LTR, however there still remains a risk that insertional mutagenesis may arise from this genetically integrated exogenous DNA [51-53].

To circumvent the integration step altogether, several groups have resorted to non-integrating viruses such as adenoviruses for reprogramming factor delivery. However, adenoviral methods are highly inefficient and limited to permissive cell types. Stadtfeld et al. reported mouse iPSCs from fibroblasts and liver cells at extremely low efficiency (0.0001% to 0.001%), which is significantly lower than that obtained from integrating viruses (0.01% to 0.1%) [18,54]. Their low efficiency has been largely attributed to the failure to sustain factor expression due to a gradual loss of viral vectors in dividing cells. Sendai virus (SeV), is another type of non-integrating virus vector which has been successfully used to reprogram human cells [55]. SeV viruses are RNA viruses and hence do not integrate into host genome unlike other DNA viruses [56-58]. Additionally, SeV viruses are replication competent and hence unlike the adenoviruses can sustain high-levels of factor expression, which can reach up to 10 times higher (efficiency ~1%) than those obtained by conventional retroviral vectors. An additional benefit of this system is the convenient removal of viruses by antibody mediated negative selection utilizing the cell surface marker, HN, expressed on SeV infected cells.

Plasmid DNA vectors for generating Transgene-free iPSCs

Several groups have successfully generated non-viral, transgene-free iPSCs using DNA-based expression vectors such as plasmid, mini circle vector, piggyBac transposon and episomal plasmid. But this transgene-free factor delivery comes at a price of low efficiency as transient transfection of DNA vectors is less efficient than viral transduction [59]. To this end, several groups have reportedly used polycistronic expression cassettes to ensure that every cell gets the full complement of factors [17,60]. Alternately, mini circle vectors expressing Oct4, Sox2, Nanog and Lin28 from a single cassette of OSNL plus a GFP reporter gene, each separated by self-cleaving peptide 2A sequences, have

been used to generate transgene-free iPSCs from human adipose stem cells [61]. These vectors used circular expression cassettes produced from regular plasmids by removing the bacterial backbone through intra molecular recombination. Compared to their parental plasmids, mini circles are smaller in size and less prone to silencing, both of which make them highly sought after for reprogramming somatic cells, however, they are far less efficient compared to retroviral vectors (reprogramming efficiency ~0.005%) [62].

Moreover, their usage requires cumbersome protocols with large number of starting cells and repeated transient transfections to compensate for the rapid loss of reprogramming factors in dividing cells. But compared to other methods of reprogramming mini circle approach has several advantages, including the use of a single vector without the need for subsequent drug selection, vector excision, or the inclusion of oncogenes such as SV40, making the derivation process free of foreign transgenes or chemicals. These unique features bagged it the FDA approval, making it a potentially significant approach from clinical and translational standpoint.

Some other groups designed a piggyBac transposon approach to deliver reprogramming factors to host cells [63-67]. In this approach, a transient transposase expression leads to stable integration of the piggyBac transposon cassette into the host genome, thus ensuring a high level of expression of reprogramming factors. Interestingly, the piggyBac transposon cassette can be easily excised, taking advantage of the natural propensity of the system for seamless excision, by transiently expressing the transposase gene in iPSCs without any risk of mutations at the integration site [68]. However, the excision step is inefficient and in certain cases reversion of iPSCs to wild type phenotypes has been observed after piggyBac transposon excision.

An alternate strategy for stable expression of reprogramming factors is made available by the use of non-integrating oriP/EBNA1-based episomal vectors [69,70]. Derived from the Epstein-Barr virus, oriP vectors can be easily transfected into human somatic cells, without the need for viral packaging, and can be subsequently removed from cells in the absence of drug selection without further genetic manipulation. In such vectors the cis element oriP and the EBNA1 gene product act in trans and together control the replication and partition of the episomes during cell cycle. This process provides these vectors with self-sufficiency to function in a variety of cell types, eliminating the need for repeated transfections. Additionally, the removal of episomal transgenes is not only automatic and efficient but also error proof, thus making oriP episomal vectors a method of choice. However, the stable transfection efficiency of the oriP vectors is several orders of magnitude less than that of the lentiviral vectors.

DNA-free methods for generating Transgene-free iPSCs

To overcome the caveat of genome modification presented by DNA-based vectors, several groups have successfully attempted to deliver reprogramming factors as mRNAs or proteins. In such cases, instead of being produced by a transgene, recombinant proteins are directly delivered to cells by fusing them to cell-penetrating peptides such as poly-arginine [71-73]. Although such proteins make it to the nucleus successfully, but require repeated application for a successful reprogramming. Moreover, all reports of protein-based reprogramming suffer from low efficiencies (~0.001%). Additionally, it is technically challenging to produce large quantities of biochemically active recombinant proteins.

Worth highlighting also is the discrepancy in the various published reports. Compared to Zhou et al. [28] who show incapability in generating mouse iPSCs with only recombinant proteins, without the use of chemical supplements, Kim et al. [29] demonstrated successful derivation of human iPSCs with direct delivery of reprogramming proteins in the absence of any chemical treatment. The plausible scientific explanation for these observed differences could be the mode of expression of reprogramming proteins, which involved mammalian cells in case of Kim et al. while expression in E.coli followed by protein refolding for Zhou et al. [28] Taken together, direct protein based reprogramming approach still remains a cumbersome technique offering poor efficiencies compared to other known approaches, thereby lacking the competitive edge to be of value in clinical setting.

As an alternative, synthetic mRNAs have shown promise for fast and efficient reprogramming [74-78]. Warren et al. [74] proposed a non-integrating strategy for cellular reprogramming by delivery of synthetic mRNA, modified to overcome innate anti-viral responses [74,79,80]. A five-factor cocktail (OKSML) was able to reprogram human fibroblasts with an efficiency of 2%, which is two orders of magnitude higher than virus-based reprogramming. Additionally, microRNAs (miRNAs) have shown great potential in regulating pluripotency [81-85]. Intriguingly, five miRNAs (miR-302a-d and miR-367) have been shown to be sufficient in reprogramming human cells without any exogenous transcription factor [86,87]. Later, another group reported Tg-free iPSCs in mouse and human systems by transfections of mature miRNAs (different combinations of miR-200c, 302s and 369s) [88]. While their ease of production and small size ensuring efficient delivery makes mRNAs and miRNAs an attractive option for the production of transgene-free iPSCs but poor efficiency still remains a stumbling block.

The quest for reprogramming somatic cells solely with the use of chemical compounds ended when Hou et al. [89] reported the derivation of mouse iPSCs at a frequency as high as ~0.2% using a combination of seven small-molecule compounds [5,19,21-23,28,42,90-96]. This discovery brought a paradigm shift to the technology of cellular reprogramming. Additionally, the use of small molecules alone brought with it several advantages like the molecules being cell permeable showing a promise of easy delivery, easily synthesized in large quantities and non-immunogenic. But while the ease of use and efficiency of these small molecules in mouse system has been well documented, their success in human cells is yet to be established.

Conclusion: Challenges Ahead

Many roadblocks remain to be addressed before iPSCs reach the clinic [97-109]. A major impediment to the use of iPSCs for therapeutic purposes remains the viral-based delivery of reprogramming factors. Additionally, for patient-specific autologous treatment, methods need to improve to be able to generate iPSCs in sufficient quantity. Efficiency of reprogramming remains the biggest challenge, especially in the context of technologies that do not integrate transgenes into the host genome. Moreover, there still remains a risk of teratoma formation in case a subpopulation of iPSCs fails to terminally differentiate

prior to transplantation.

Even upon differentiation it is difficult to control and predict events of spontaneous differentiation and dedifferentiation into other cell types and the clinical outcome of such a heterologous cell populations within a certain tissue. Current scientific and technical advancements are rapidly eliminating some of these fundamental issues in the iPSC field thereby reassuring the profound benefits of stem cell technology to regenerative medicine. But while a lot of ground has been covered, there might be a long way to go ahead before the full potential of iPSC technology may be harnessed.

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References

1. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4): 663-676.
2. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, et al. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131(5): 861-872.
3. Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, et al. (2007) In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature 448(7151): 318-324.
4. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, et al. (2007) Induced pluripotent Stem Cell lines derived from human somatic cells. Science 318(5858): 1917-1920.
5. Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, et al. (2008) Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nature biotechnology 26(7): 795-797.
6. Maherali N, Ahfeldt T, Rigamonti A, Utikal J, Cowan C, et al. (2008) A high-efficiency system for the generation and study of human induced pluripotent stem cells. Cell Stem Cell 3(3): 340-345.
7. Maherali N, Hochedlinger K (2008) Guidelines and techniques for the generation of induced pluripotent stem cells. Cell Stem Cell 3(6): 595-605.
8. Park IH, Zhao R, West JA, Yabuuchi A, Huo H, et al. (2008) Reprogramming of human somatic cells to pluripotency with defined factors. Nature 451(7175): 141-146.
9. Zhao Y, Yin X, Qin H, Zhu F, Liu H, et al. (2008) Two supporting factors greatly improve the efficiency of human iPSC generation. Cell Stem Cell 3(5): 475-479.
10. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, et al. 1998. Embryonic Stem Cell lines derived from human blastocysts. Science 282(5391): 1145-1147.
11. Blelloch R, Venere M, Yen J, Ramalho-Santos M (2007) Generation of induced pluripotent stem cells in the absence of drug selection. Cell Stem Cell 1(3): 245-247.
12. Okita K, Ichisaka T, Yamanaka S (2007) Generation of germline-competent induced pluripotent stem cells. Nature 448(7151): 313-317.
13. Hockemeyer D, Soldner F, Cook EG, Gao Q, Mitalipova M, et al. (2008) A drug-inducible system for direct reprogramming of human somatic cells to pluripotency. Cell Stem Cell 3(3): 346-353.
14. Huangfu D, Osafune K, Maehr R, Guo W, Eijkelenboom A, et al. (2008) Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. Nature Biotechnology 26(11): 1269-1275.
15. Kim JB, Zaehres H, Wu G, Zentile L, Ko K, et al. (2008) Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. Nature 454(7204): 646-650.
16. Nakagawa M, Koyanagi M, Zanabe K, Takahashi K, Ichisaka T, et al. (2008) Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nature Biotechnology 26(1): 101-106.
17. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S (2008) Generation of mouse induced pluripotent stem cells without viral vectors. Science 322(5903): 949-953.
18. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K (2008) Induced pluripotent stem cells generated without viral integration. Science 322(5903): 945-949.
19. Feng B, Ng JH, Heng JC, Ng HH (2009) Molecules that promote or enhance reprogramming of somatic cells to induced pluripotent stem cells. Cell Stem Cell 4(4): 301-312.
20. Ichida JK, Blanchard J, Lam K, Son EY, Chung JE, et al. (2009) A small-molecule inhibitor of tgf-Beta signaling replaces sox2 in reprogramming by inducing nanog. Cell Stem Cell 5(5): 491-503.
21. Li W, Wei W, Zhu S, Zhu J, Shi Y, et al. (2009) Generation of rat and human induced pluripotent stem cells by combining genetic reprogramming and chemical inhibitors. Cell Stem Cell 4(1): 16-19.
22. Lin T, Ambasudhan R, Yuan X, Li W, Hilcove S, et al. (2009) A chemical platform for improved induction of human iPSCs. Nature Methods 6(11): 805-808.
23. Lyssiotis CA, Foreman RK, Staerk J, Garcia M, Mathur D, et al. (2009) Reprogramming of murine fibroblasts to induced pluripotent stem cells with chemical complementation of Klf4. Proc Natl Acad Sci U S A 106(22): 8912-8917.
24. Miura K, Okada Y, Aoi T, Okada A, Takahashi K, et al. (2009) Variation in the safety of induced pluripotent Stem Cell lines. Nature Biotechnology 27(8): 743-745.
25. Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, et al. (2010) Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell 142(3): 375-386.
26. Nakagawa M, Takizawa N, Narita M, Ichisaka T, Yamanaka T (2010) Promotion of direct reprogramming by transformation-deficient Myc. Proc Natl Acad Sci U S A 107(32): 14152-14157.
27. Rodriguez-Piza I, Richaud-Patin Y, Vassena R, Gonzalez F, Barrero MJ, et al. (2010) Reprogramming of human fibroblasts to induced pluripotent stem cells under xeno-free conditions. Stem Cells 28(1): 36-44.
28. Zhu S, Li W, Zhou H, Wei W, Ambasudhan R, et al (2010) Reprogramming of human primary somatic cells by OCT4 and chemical compounds. Cell Stem Cell 7(6): 651-655.
29. Lee CH, Kim JH, Lee HJ, Jeon K, Lim H, et al. (2011) The generation of iPS cells using non-viral magnetic nanoparticle based transfection. Biomaterials 32(28): 6683-6691.
30. Awe JP, Lee PC, Ramathal C, Vega-Crespo A, Durruthy-Durruthy, et al. (2013) Generation and characterization of transgene-free human induced pluripotent stem cells and conversion to putative clinical-grade status. Stem Cell Research & Therapy 4(4): 87.

31. Chen W, Tsai PH, Hung Y, Chiou SH, Mou CY (2013) Nonviral cell labeling and differentiation agent for induced pluripotent stem cells based on mesoporous silica nanoparticles. ACS Nano 7(10): 8423-8440.

32. Habib O, Habib G, Choi HW, Hong KS, Do JT, et al. (2013) An improved method for the derivation of high quality iPSCs in the absence of c-Myc. Experimental Cell Research 319(20): 3190-3200.

33. Kishino Y, Seki T, Fujita J, Yuasa S, Tohyama S, et al. (2014) Derivation of transgene-free human induced pluripotent stem cells from human peripheral T cells in defined culture conditions. PloS one 9(5): e97397.

34. Lu HF, Chai C, Lim TC, Leong MF, Lim JK, et al. (2014) A defined xeno-free and feeder-free culture system for the derivation, expansion and direct differentiation of transgene-free patient-specific induced pluripotent stem cells. Biomaterials 35(9): 2816-2826.

35. Raab S, Klingenstein M, Liebau S, Linta L (2014) A Comparative View on Human Somatic Cell Sources for iPSC Generation. Stem Cells Int 2014: 768391.

36. Hochedlinger K, Jaenisch R (2006) Nuclear reprogramming and pluripotency. Nature 441(7097): 1061-1067.

37. Eminli S, Utikal J, Arnold K, Zaenisch R, Hochedlinger K (2008) Reprogramming of neural progenitor cells into induced pluripotent stem cells in the absence of exogenous Sox2 expression. Stem Cells 26(10): 2467-2474.

38. Marson A, Foreman R, Chevalier B, Bilodeau S, Kahn M, et al. (2008) Wnt signaling promotes reprogramming of somatic cells to pluripotency. Cell Stem Cell 3(2): 132-135.

39. Stadtfeld M, Maherali N, Breault NT, Hochedlinger K (2008) Defining molecular cornerstones during fibroblast to iPS cell reprogramming in mouse. Cell Stem Cell 2(3): 230-240.

40. Daley GQ, Lensch MW, Jaenisch R, Meissner A, Plath K, et al. (2009) Broader implications of defining standards for the pluripotency of iPSCs. Cell Stem Cell 4(3): 200-201; author reply 202.

41. Hanna J, Saha K, Pando B, Zan Zon J, Lengner CJ, et al. (2009) Direct cell reprogramming is a stochastic process amenable to acceleration. Nature 462(7273): 595-601.

42. Yoshida Y, Takahashi K, Okita K, Ichisaka T, Yamanaka S (2009) Hypoxia enhances the generation of induced pluripotent stem cells. Cell Stem Cell 5(3): 237-241.

43. Boue S, Paramonov I, Barrero MJ, Izpisua Belmonte JC (2010) Analysis of human and mouse reprogramming of somatic cells to induced pluripotent stem cells. What is in the plate? PloS One 5(9): e12664.

44. Malik N, Rao MS (2013) A review of the methods for human iPSC derivation. Methods in Molecular Biology 997: 23-33.

45. Graversen VK, Chavala SH (2014) Induced Pluripotent Stem Cells: Generation, Characterization, and Differentiation-Methods and Protocols. Methods mol biol.

46. Liu K, Song Y, Yu H, Zhao T (2014) Understanding the roadmaps to induced pluripotency. Cell Death & Disease 5: e1232.

47. Carey BW, Markoulaki S, Hanna J, Saha K, Gao G, et al. (2009) Reprogramming of murine and human somatic cells using a single polycistronic vector. Proc Natl Acad Sci U S A 106(1): 157-162.

48. Chang CW, Lai YS, Pawlik KM, Liu K, Sun CW, et al. (2009) Polycistronic lentiviral vector for "hit and run" reprogramming of adult skin fibroblasts to induced pluripotent stem cells. Stem cells 27(5): 1042-1049.

49. Soldner F, Hockemeyer D, Beard C, Gao Q, Bell GW, et al. (2009) Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 136(5): 964-977.

50. Sommer CA, Stadtfeld M, Murphy GJ, Hochedlinger K, Kotton DN, et al. (2009) Induced pluripotent Stem Cell generation using a single lentiviral Stem Cell cassette. Stem cells 27(3): 543-549.

51. Papapetrou EP, Sadelain M (2011) Generation of transgene-free human induced pluripotent stem cells with an excisable single polycistronic vector. Nat Protoc 6(9): 1251-1273.

52. Papapetrou EP, Sadelain M (2011) Derivation of genetically modified human pluripotent stem cells with integrated transgenes at unique mapped genomic sites. Nat Protoc 6(9): 1274-1289.

53. Kuehle J, Turan S, Cantz T, Hoffmann D, Suerth JD, et al. (2014) Modified lentiviral LTRs allow Flp recombinase-mediated cassette exchange and in vivo tracing of "factor-free" induced pluripotent stem cells. Mol ther 22(5): 919-928.

54. Zhou W, Freed CR (2009) Adenoviral gene delivery can reprogram human fibroblasts to induced pluripotent stem cells. Stem Cells 27(11): 2667-2674.

55. Fusaki N, Ban H, Nishiyama A, Saeki k, Hasegawa M (2009) Efficient induction of transgene-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 85(8): 348-362.

56. Li HO, Zhu YF, Asakawa M, Kuma H, Hirata T, et al. (2000) A cytoplasmic RNA vector derived from nontransmissible Sendai virus with efficient gene transfer and expression. J Virol 74(14): 6564-6569.

57. Lieu PT, Fontes A, Vemuri MC, Macarthur CC (2013) Generation of induced pluripotent stem cells with CytoTune, a non-integrating Sendai virus. Methods Mol Biol 997: 45-56.

58. Fujie Y, Fusaki N, Katayama T, Hamasaki M, Soejima Y, et al. (2014) New type of Sendai virus vector provides transgene-free iPS cells derived from chimpanzee blood. PloS One 9(12): e113052.

59. Okita K, Hong H, Takahashi K, Yamanaka S (2010) Generation of mouse-induced pluripotent stem cells with plasmid vectors. Nature Protocols 5(3): 418-428.

60. Gonzalez F, Barragan Monasterio M, Tiscornia G, Montserrat Pulido N, Vassena R, et al. (2009) Generation of mouse-induced pluripotent stem cells by transient expression of a single nonviral polycistronic vector. Proc Natl Acad Sci U S A 106(22): 8918-8922.

61. Jia F, Wilson KD, Sun N, Gupta DM, Huang M, et al. (2010) A nonviral minicircle vector for deriving human iPS cells. Nature Methods 7(3): 197-199.

62. Chen ZY, He CY, Kay MA (2005) Improved production and purification of minicircle DNA vector free of plasmid bacterial sequences and capable of persistent transgene expression in vivo. Hum Gene Ther 16(1): 126-131.

63. Yusa K, Rad R, Takeda J, Bradley A (2009) Generation of transgene-free induced pluripotent mouse stem cells by the piggyBac transposon. Nat Methods 6(5): 363-369.

64. Woltjen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, et al. (2009) piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 458(7239): 766-770.

65. Kaji K, Norrby K, Paca A, Mileikovsky M, Mohseni P, et al. (2009) Virus-free induction of pluripotency and subsequent excision of reprogramming factors. Nature 458(7239): 771-775.

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66. Wilson MH, Coates CJ, George AL (2007) PiggyBac transposon-mediated gene transfer in human cells. Mol ther 15(1): 139-145.

67. Davis RP, Nemes C, Varga E, Freund C, Kosmidis G, et al. (2013) Generation of induced pluripotent stem cells from human foetal fibroblasts using the Sleeping Beauty transposon gene delivery system. Differentiation; Research In Biological Diversity 86(1-2): 30-37.

68. Lacoste A, Berenshteyn F, Brivanlou AH (2009) An efficient and reversible transposable system for gene delivery and lineage-specific differentiation in human embryonic stem cells. Cell Stem Cell 5(3): 332-342.

69. Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, et al. (2009) Human induced pluripotent stem cells free of vector and transgene sequences. Science 324(5298): 797-801.

70. Fontes A, Macarthur CC, Lieu PT, Vemuri MC (2013) Generation of human-induced pluripotent stem cells (hiPSCs) using episomal vectors on defined Essential 8 Medium conditions. Methods Mol Biol 997: 57-72.

71. Zhou H, Wu S, Joo JY, Zhu S, Han DW, et al. (2009) Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 4(5): 381-384.

72. Kim D, Kim CH, Moon JI, Chung YG, Chang MH, et al. (2009) Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell 4(6): 472-476.

73. Cho HJ, Lee CS, Kwon, Paek JS, Lee SH, et al. (2010) Induction of pluripotent stem cells from adult somatic cells by protein-based reprogramming without genetic manipulation. Blood 116(3): 386-395.

74. Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, et al. (2010) Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell 7(5): 618-630.

75. Li M, Sancho-Martinez I, Izpisua Belmonte JC (2011) Cell fate conversion by mRNA. Stem Cell Res Ther 2(1): 5.

76. Li Z, Yang CS, Nakashima K, Rana TM (2011) Small RNA-mediated regulation of iPS cell generation. EMBO J 30(5): 823-834.

77. Bernal JA (2013) RNA-based tools for nuclear reprogramming and lineage-conversion: towards clinical applications. J Cardiovasc Trans Res 6(6): 956-968.

78. Yoshioka N, Gros E, Li HR, Kumar S, Deacon DC, et al. (2013) Efficient generation of human iPSCs by a synthetic self-replicative RNA. Cell Stem Cell 13(2): 246-254.

79. Warren L, Ni Y, Wang J, Guo X (2012) Feeder-free derivation of human induced pluripotent stem cells with messenger RNA. Sci Rep 2: 657.

80. Mandal PK, Rossi DJ (2013) Reprogramming human fibroblasts to pluripotency using modified mRNA. Nat Protoc 8(3): 568-582.

81. Judson RL, Babiarz JE, Venere M, Blelloch R (2009) Embryonic stem cell-specific microRNAs promote induced pluripotency. Nature Biotechnology 27(5): 459-461.

82. Mallanna SK, Rizzino A (2010) Emerging roles of microRNAs in the control of embryonic stem cells and the generation of induced pluripotent stem cells. Dev Biol 344(1): 16-25.

83. Subramanyam D, Lamouille S, Judson RL, Liu JY, Bucay N, et al. (2011) Multiple targets of miR-302 and miR-372 promote reprogramming of human fibroblasts to induced pluripotent stem cells. Nat Biotechnol 29(5): 443-448.

84. Wang T, Shi SB, Sha HY (2013) MicroRNAs in regulation of pluripotency and somatic cell reprogramming: small molecule with big impact. RNA Biol 10(8): 1255-1261.

85. Wang T, Warren ST, Jin P (2013) Toward pluripotency by reprogramming: mechanisms and application. Protein Cell 4(11): 820-832.

86. Anokye-Danso F, Trivedi CM, Juhr D, Gupta M, Cui Z, et al. (2011) Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell 8(4): 376-388.

87. Liao B, Bao X, Liu L, Feng S, Zovoilis A, et al. (2011) MicroRNA cluster 302-367 enhances somatic cell reprogramming by accelerating a mesenchymal-to-epithelial transition. J Biol Chem 286(19): 17359-17364.

88. Miyoshi N, Ishii H, Nagano H, Haraguchi N, Dewi DL, et al. (2011) Reprogramming of mouse and human cells to pluripotency using mature microRNAs. Cell Stem Cell 8(8): 633-638.

89. Hou P, Li Y, Zhang X, Liu C, Guan J, et al. (2013) Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. Science 341(6146): 651-654.

90. Desponts C, Ding S (2010) Using small molecules to improve generation of induced pluripotent stem cells from somatic cells. Methods in Molecular Biology 636: 207-218.

91. Esteban MA, Wang T, Qin B, Yang J, Qin D, et al. (2010) Vitamin C enhances the generation of mouse and human induced pluripotent stem cells. Cell Stem Cell 6(1): 71-79.

92. Pasha Z, Haider H, Ashraf M (2011) Efficient non-viral reprogramming of myoblasts to stemness with a single small molecule to generate cardiac progenitor cells. PloS one 6(8): e23667.

93. Li Z, Rana TM (2012) A kinase inhibitor screen identifies small-molecule enhancers of reprogramming and iPS cell generation. Nat Commun 3: 1085.

94. Trokovic R, Weltner J, Manninen T, Mikkola M, Lundin K, et al. (2013) Small molecule inhibitors promote efficient generation of induced pluripotent stem cells from human skeletal myoblasts. Stem Cells Dev 22(1): 114-123.

95. Zhang R, Zhang LH, Xie X (2013) iPSCs and small molecules: a reciprocal effort towards better approaches for drug discovery. Acta Pharmacol Sin 34(6): 765-776.

96. Wei X, Chen Y, Xu Y, Zhan Y, Zhang R, et al. (2014) Small molecule compound induces chromatin de-condensation and facilitates induced pluripotent Stem Cell generation. J Mol Cell Biol 6(5): 409-420.

97. Lu X, Zhao T (2013) Clinical therapy using iPSCs: hopes and challenges. Genomics Proteomics Bioinformatics 11(5): 294-298.

98. Rao M (2013) iPSC-Based cell therapy: an important step forward. Stem Cell Reports 1(4): 281-282.

99. Rao M (2013) iPSC crowdsourcing: a model for obtaining large panels of Stem Cell lines for screening. Cell Stem Cell 13(4): 389-391.

100. Shtrichman R, Germanguz I, Itskovitz-Eldor J (2013) Induced pluripotent stem cells (iPSCs) derived from different cell sources and their potential for regenerative and personalized medicine. Curr Mol Med 13(5): 792-805.

101. Wu M, Chen G, Hu B (2013) Induced pluripotency for translational research. Genomics Proteomics Bioinformatics 11(5): 288-293.

102. Abdelalim EM, Bonnefond A, Bennaceur-Griscelli A, Froguel P (2014) Pluripotent stem cells as a potential tool for disease modelling and

Citation: Shweta B, Gongxiong W (2015) Induced Pluripotent Stem-cell Lines in the Clinic - Still a Long Road Ahead. J Hum Virol Retrovirol 2(3): 00044. DOI: 10.15406/jhvrv.2015.02.00044

cell therapy in diabetes. Stem Cell rev 10(3): 327-337.

103. Csobonyeiova M, Polak S, Koller J, Danisovic L (2014) Induced pluripotent stem cells and their implication for regenerative medicine. Cell Tissue Bank.

104. Inoue H, Nagata N, Kurokawa H, Yamanaka S (2014) iPS cells: a game changer for future medicine. EMBO J 33(5): 409-417.

105. Kim C (2014) iPSC technology- Powerful hand for disease modeling and therapeutic screen. BMB Rep.

106. Ko HC, Gelb BD (2014) Concise review: drug discovery in the age of the induced pluripotent stem cell. Stem Cells Transl Med 3(4): 500-509.

107. Sivapatham R, Zeng X (2014) Generation and Characterization of Patient-Specific Induced Pluripotent Stem Cell for Disease Modeling. Methods Mol Biol.

108. Walmsley GG, Hyun J, McArdle A, Senarath-Yapa K, Hu MS, et al. (2014) Induced pluripotent stem cells in regenerative medicine and disease modeling. Curr Stem Cell Res Ther 9(2): 73-81.

109. Santostefano KE, Hamazaki T, Biel NM, Jin S, Umezawa A, et al. (2015) A practical guide to induced pluripotent Stem Cell research using patient samples. Lab Invest 95(1): 4-13.

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