Delivering Factors for Reprogramming a Somatic Cell to Pluripotency

Soong Ho Um

Department of Chemical Engineering, Sungkyunkwan University, Seoul, Korea

An adult cell originates from stem cell. The stem cell is usually categorized into three species including an embryonic stem cell (ESc), an adult stem cell, and an induced stem cell (iPSc). iPSc features pluripotency, which is meant to be differentiated into any types of cells. Accordingly, it is much attractive to anyone who pursuit a regenerative medicine, owing to the potential almighty. They are simply produced by reprogramming a somatic cell via a transfer of transcription factors. The efficiency and productivity of iPSc are considerably subject to delivering methods of exogenous genes into a variety of targeted mammals. Conventional and well-run gene delivery techniques have been reviewed here. This details the methods and principles of delivery factors and provides an overview of the research, with an emphasis on their potential for use as clinical therapeutic platforms.

Keywords: Induced stem cell, Delivery vector

Overview

Over a decade, several cellular engineering techniques have been developed. They were practically applied to the cutting-edge medical issues, together with the special health-care purposes for human beings. Most recently, a few of frontier researchers at the scientific boundaries have strongly desired to find new way about reprogramming any eukaryotes with a pluripotency. It is currently revealed to be an induced pluripotent stem cell technology (iPSc technology). As compared with conventional and analogous stem cell engineering, which includes the derivation of ES cells from the inner cell mass of the pre-implantation embryo at the blastocyst stage, the fusion of somatic cells with pre-existing ES cells and derivation of ES cells from embryos generated by nuclear transfer, it has

been rather striking in that any cell types can be converted to pluripotent cell. It is highly expected that damaged tissues may be easily replaced or repaired with fresh ones (Fig. 1). Undeniably, it is now exciting many investigators who are studying a regenerative and a developmental evolution.

En route for the pluripotent cell differentiation have been attempted. The first was achieved by Shinya Yamanaka and his colleagues. They have used a mouse fibroblast as a proof-of-concept model cell, in which it was presented as the retroviral-introduction of four different transcription factors including octamer-binding transcription factor-3/4 (OCT3/4), SRY-related high-mobility-group (HMG)-box protein-2 (SOX2), MYC and Kruppel-like factor-4 (KLF4) induced pluripotency (1). Later, their technique has been broadly expanded into other types of cells containing mouse cells (2) and human somatic cells (3-5). Besides the complexes of four original transcription factors described above, current studies have interestingly demonstrated that other combinations of substitutes for the original transcription factors can produce iPSc cells. For example, some researchers have substituted MYC and KLF4 with other candidates such as NANOG and LIN28 (6, 7) as OS is remained as basic

Accepted for publication January 2, 2012

Correspondence to **Soong Ho Um**

Department of Chemical Engineering, Sungkyunkwan University,
300 Cheonchoen-dong, Jangan-gu, Suwon 440-746, Korea

Tel: +82-31-290-7348, Fax: +82-31-290-7272

E-mail: sh.um@skku.edu

Fig. 1. Generation of induced pluripotent stem cell reprogrammed from somatic cell via delivery factors.

resources. Most recently, Sinya and his colleagues have replaced one of the transcription factors with Glis 1. It is evaluated to be a realistically agreeable substitute against MYC and KLF4 since it is proved to be a non-cancerous induced gene as reported (8). Nowadays, many candidates containing Esrrb and SV40 LT p53 siRNA have been reported to promote or enhance reprogramming of somatic cells. Small chemicals and their complexes have been also deliberated. They can enhance or alleviate the reprogramming efficiency, by replacing the core factors. In addition to the transcription factors, pluripotent reprogramming is varied depending on the cell types and delivery methods under special culture conditions. The reprogramming menu is briefly shown in the Fig. 2. If you are more interested in a variety of inducing factors such as transcription factors described above or any conditions including a cell incubation time or environmental resources for producing iPSCs, which are beyond the scope of this review, you can alternatively refer to other literatures (9, 10).

In this text, it is mainly emphasized that a delivery factor is the utmost of principal factors affecting the pluripotency. The delivery is stated to be a cellular uptake of exogenous genes through plasma membrane and an inclusion in the chromosome via nuclear pore transport so as to integrate genomic system. Various delivery systems have been described together with the working principles.

Generally, a gene delivery is assumed to directly inject the encoding genes into the cell. The direct gene injection

is simple and appealing and straightforward. It is also less-contaminant but includes certain risks (e.g., it may happen that the gene isn't expressed or overexpressed) and rather slow, laborious and one at a time. Moreover, the gene molecules with higher molecular weight and negative charge are usually prevented by the target cell due to the charge repulsion. Accordingly, it is necessarily required to have any protecting materials. Two systems are mainly categorized: viral system and non-viral system.

A target gene for iPSc is usually delivered by a viral transduction as did a traditional gene therapy. In present, it is moving fast to the non-viral vector systems excluding virus molecules as a carrier. Such trend may be owed to the following proofs; the virus-mediated delivery system is announced to have a perpetual threat regarding 1) the transgene integration into the host genome and 2) the oncogenicity of two transcription factors termed as Klf4 and c-Myc. To avoid the severe side effects and transform into iPSc, novel delivery tool kits should be available on demand. Recently, a polymer based delivery system considering safety issues at the priority have been highlighted even though it suffers from lower delivery efficiency. To improve it, topical studies are now moving through the investigation of the gene transfer pathways through cellular membrane barriers. Synthetic DNA delivery systems have been comprehensively reviewed as described by reference (11). iPSc transcription factors are transported into the internal cellular membrane through several obstacles

Cell types		Transcription factors		Delivery modes	
Ease of reprogramming	Fibroblasts, T cells, Neural stem cells	Importance for embryonic development	OCT4, SOX2, NANOG, UTF1, LIN28, SALL4, NR5A2, TBX3, ESSRB, DPPA4	Efficiency (low)	Naked DNA, Adenovirus
Middle	Keratinocytes, Adipose stem cells, Mesenchymal stem cells, Dental pulp stem cells, Hepatocytes, Amniotic fluid cells	Importance for proliferation and cell cycle	MYC, KLF4, SV40LT, REM2, MDM2, cyclin D1	Middle	Electrical, Chemical, Mechanical methods, Polymer based system
Hard	Cord blood cells, Germline stem cells			High	Microinjection, MMLV-derived retrovirus, Lentivirus, Excisable lentivirus

Fig. 2. Representative transcription factors for reprogramming.

represented as 1) gene complexation, 2) cellular uptake, 3) endosomal escape, and 4) nuclear transport. The gene delivered is finally integrated with the host genome and decoded into the target proteins necessary for reprogramming. In the end, a cellular uptake and a nuclear transport are mainly considered to be a crucial hurdle for gaining the pluripotent cell. To achieve it, a number of delivery methods have been suggested. They are approaching to the current stem cell based studies; leading to the regenerative medicines (12). Two strategies for the cellular uptake are presented here.

Virus based (or virus-mimic, transduction) delivery system

Basically, a virus based delivery vehicle is prepared by removing infection factors that virus has inside. Viruses possess superior properties capable of infecting a host material but together with much stronger viral toxicity such as cancerous or disease-encouraging abilities even though recently, many attempts to attenuate the toxicity have been made on clinical trials. As a representative, retrovirus, adenovirus and adeno-associated viruses (AAV) have been well known. In case of the retrovirus-mediated delivery method, Moloney murine leukaemia virus (MMLV)-derived retroviruses such as pMXs, pLib, or pMSCV are indicated. They selectively infect the cell, while making copies during the cell division. Most importantly, they are

able to carry exogenous genes safely into host genome and resultantly, making target genes expressed. However, they suffers from non-specific anchoring of the genes on the chromosome, finally leading to the inducing multiple mutations. In order to decrease the possibility of insertional mutagenesis, many trials have been suggested but not in success (13, 14). Different from the retrovirus system, adenovirus is the characteristic of delivering genes into multi-types of cells including dividing and non-dividing cells. It is also able to carry genes with a larger molecular weight (15, 16). However, its use is hesitated by the induction of a temporary genetic expression and immunogenicity. Recently, advanced recombinant technologies have been suggested so as to solve the problems on the virus samples described above (17, 18).

Last, adeno-associated viruses (AAV) are not relevant to any types of infection diseases. Therefore, it may make AAV popular and be safely used as a gene-carrier. Accordingly, it is fairly useful for stem cell studies. Human or mouse-derived ESCs (19), MSCs and various stem cell lines are likely to be handled together with AAV techniques. AAV has no immunogenicity as different from the previous vectors. However, it suffers from the very restricted gene size, which maximizes up to 5.2 kb.

Non-viral vector system

Nonviral vector (transfection) systems have been most

recently underlined, becoming the great replacement of viral vector system. Commonly, viral vector systems have been suffering from its intrinsic toxicity and high risk of genome contamination. Relatively, non-viral vector systems have been aiming at non-toxic and less risky ones but much efficient (11, 20). Primarily, all systems not using any virus are included. According to the rational design of a polymer, systematic complexation for efficient uptake may be completed. A variety of non-viral delivery methods have been summarized in the Figure 3. Physical, mechanical, and chemical (polymeric) approaches have been developed. The delivery efficiency of each system is compared, together with the toxicity evaluation.

Mechanical and electrical methods

In terms of the mechanical gene delivery system, it demonstrates the injection of gene molecules by pressure drop or particle bombardment. Besides the direct injection of naked DNA molecules or pressure-mediated transfection, traditionally, DNA was delivered to mammalian cells through continuous high-voltage electrical pulses, which is called as electroporation. It is much limited because of an induced cell death after higher voltage exposure. Alternatively, it is further used to a gene therapy via in-skin, in-corneal endothelium, in-muscle (21-23).

Chemical (polymer based) methods

Since the early 1970's, several polymer groups have proved to be a versatile delivery vehicle for exogenous gene transport. It basically goes after the principle regard-

ing the complexation between DNA and positively charged moiety on the polymeric backbone or its functional groups. It is classified by synthetic polymers (e.g., PEI or PAMAM dendrimer) and natural biopolymers (e.g., protein, polypeptide, lipid). Fig. 3 shows representative polymer groups and compares them in the respect of toxicity and efficiency. Before the initial introduction of polymer groups, a small and cationic molecule such as DEAE-dextran or calcium phosphate was first used for the entry of DNA complexes. It however suffered from the unpredictable complexation caused by intermolecular interaction of the small cations and it was convinced to be a huge immunogenicity. Next, DNA construct was mixed with various lipid components including cationic, neutral lipids. Lipid based systems are exceedingly expected to be very compatible with cellular membranes, owing to the compositional features replicating the plasma membrane (24). However, it was evident that the system was deficient in active targeting once mixed with variable sizes of DNA molecules. Polymer based system can systematically tune the charge imbalance and alleviate the aggregation. Polyethylenimine (PEI) was a representative of cationic polymers used for a long time. It effectively condenses DNA with cationic charged polymeric nanoparticle and then brings into cellular membrane via endocytosis pathways. It results in nuclear transport of free DNA right after the swelling of the trapped endosome in a cytosol (25). However, it was greatly recognized that it rather has cytotoxicity at the threshold value of 25,000 g per mol (26). Mikos and his colleagues have investigated the intracellular pathways for PEI-DNA complexes (27).

Alternatively, natural polymers such as poly-L-lysine are advocated, which are produced by bacterial fermentation or artificially synthesized. The vast amount of its positive-charges envelope negative charged DNA, thus leading to a particulate form with nano or micro scale.

Fig. 3. Delivery efficiency versus toxicity for various delivery methods.

Perspectives

In addition to the cellular uptake described, nuclear targeting and chromosomal integration have been thoroughly studied and addressed. Inspired by the educational instruction from viral systems, viral nuclear signals have been fused with DNA molecules so as to induce the higher delivery efficiency. NLS (viral nuclear localization signals) are logically added to the synthetic DNA delivery system (28, 29). Most recently, nuclear targeting peptide scaffolds have been conjugated with lipid molecules and such lipid based transfection system has been applied into non-divid-

ing mammalian cells. The delivery efficiency was increased up to 80% and reporter gene expression was improved up to approximately 63 fold, compared with the previous reports (30).

Chromosomal integration has also been seriously considered. We must pursue our studies toward how to put the genes at the right position of the chromosome sequences. Exceptionally and as an attempt to enhance the stable integration of non-viral vectors, Piggybox systems have been suggested by Koji and his colleagues. It has been simply described that the huge piggybox is constructed in order to deliver vector molecules (31).

In the near future, these key players presented by now are to be well handled under the aspect of: 1) delivery efficiency and 2) genomic integration. Transgene integration method has shown an efficient delivery but faced many problems like incomplete reprogramming and tumor genesis. The vast numbers of new strategies have been attempted so as to overcome two tradeoffs to induce the reprogrammed iPS cells. The Wu group at Stanford University has made a significant footprint on the performance of proteomic characterization of iPS cells (32). Such trials make us find the optimal solutions for engineering toward iPS.

Owing to many restrictions caused by the direct integration of the gene fragments into chromosome, RNA or protein instead of DNA has been exceptionally deliberated. It has no concern about chromosomal integration of genes. Warren et al. has attempted to deliver synthetic mRNAs into somatic donor cells in order to convert it into iPSCs (33). Some polycationic materials have been combined together with protein materials via chemical or physical methods. Zhou et al. and Kim et al. delivered the protein molecules encoding OSKM directly to the target cells (34).

The researches toward iPS technology are getting popular due to its unceasing possibility to clinical trials for regenerative medicines. However, it must overcome so many obstacles including immunogenicity before directly adjusting the technology to human being for therapeutic or health-care purpose. To find the solution, several factors appointed in this review should be thoroughly investigated. Particularly, new types of vector delivery methods might be a principal solution.

Acknowledgments

We specially thank to Professor D. I. Kim for his passionate advices and encouragements. Work in the laboratory of S. H. Um was supported by grants from the National Research Foundation of Korea (NRF) funded by the Korea government (MEST) (No. 20100007782 (Mid-ca-

reer Researcher Program)) and from the Korea Health technology R&D project, Ministry of Health & Welfare, Republic of Korea (A110552).

Potential conflict of interest

There is no conflict of interest in this article.

References

1. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:663-676
2. Aoi T, Yae K, Nakagawa M, Ichisaka T, Okita K, Takahashi K, Chiba T, Yamanaka S. Generation of pluripotent stem cells from adult mouse liver and stomach cells. *Science* 2008;321:699-702
3. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007;131:861-872
4. Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, Daley GQ. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 2008;451:141-146
5. Lowry WE, Richter L, Yachechko R, Pyle AD, Tchieu J, Sridharan R, Clark AT, Plath K. Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proc Natl Acad Sci U S A* 2008;105:2883-2888
6. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007;318:1917-1920
7. Belloch R, Venere M, Yen J, Ramalho-Santos M. Generation of induced pluripotent stem cells in the absence of drug selection. *Cell Stem Cell* 2007;1:245-247
8. Maekawa M, Yamaguchi K, Nakamura T, Shibukawa R, Kodanaka I, Ichisaka T, Kawamura Y, Mochizuki H, Goshima N, Yamanaka S. Direct reprogramming of somatic cells is promoted by maternal transcription factor Glis1. *Nature* 2011;474:225-229
9. González F, Boué S, Izpisua Belmonte JC. Methods for making induced pluripotent stem cells: reprogramming à la carte. *Nat Rev Genet* 2011;12:231-242
10. Feng B, Ng JH, Heng JC, Ng HH. Molecules that promote or enhance reprogramming of somatic cells to induced pluripotent stem cells. *Cell Stem Cell* 2009;4:301-312
11. Luo D, Saltzman WM. Synthetic DNA delivery systems. *Nat Biotechnol* 2000;18:33-37
12. Wu SM, Hochedlinger K. Harnessing the potential of induced pluripotent stem cells for regenerative medicine. *Nat Cell Biol* 2011;13:497-505
13. Katz RA, Merkel G, Skalka AM. Targeting of retroviral integrase by fusion to a heterologous DNA binding domain: in vitro activities and incorporation of a fusion protein into viral particles. *Virology* 1996;217:178-190

14. Bushman FD, Miller MD. Tethering human immunodeficiency virus type 1 preintegration complexes to target DNA promotes integration at nearby sites. *J Virol* 1997;71:458-464
15. Smith AE. Viral vectors in gene therapy. *Annu Rev Microbiol* 1995;49:807-838
16. Verma IM, Somia N. Gene therapy-promises, problems and prospects. *Nature* 1997;389:239-242
17. Fleury S, Driscoll R, Simeoni E, Dudler J, von Segesser LK, Kappenberger L, Vassalli G. Helper-dependent adenovirus vectors devoid of all viral genes cause less myocardial inflammation compared with first-generation adenovirus vectors. *Basic Res Cardiol* 2004;99:247-256
18. Wen S, Graf S, Massey PG, Dichek DA. Improved vascular gene transfer with a helper-dependent adenoviral vector. *Circulation* 2004;110:1484-1491
19. Smith-Arica JR, Thomson AJ, Ansell R, Chiorini J, Davidson B, McWhir J. Infection efficiency of human and mouse embryonic stem cells using adenoviral and adeno-associated viral vectors. *Cloning Stem Cells* 2003;5:51-62
20. Pack DW, Hoffman AS, Pun S, Stayton PS. Design and development of polymers for gene delivery. *Nat Rev Drug Discov* 2005;4:581-593
21. Banga AK, Prausnitz MR. Assessing the potential of skin electroporation for the delivery of protein- and gene-based drugs. *Trends Biotechnol* 1998;16:408-412
22. Oshima Y, Sakamoto T, Yamanaka I, Nishi T, Ishibashi T, Inomata H. Targeted gene transfer to corneal endothelium in vivo by electric pulse. *Gene Ther* 1998;5:1347-1354
23. Aihara H, Miyazaki J. Gene transfer into muscle by electroporation in vivo. *Nat Biotechnol* 1998;16:867-870
24. Madeira C, Mendes RD, Ribeiro SC, Boura JS, Aires-Barros MR, da Silva CL, Cabral JM. Nonviral gene delivery to mesenchymal stem cells using cationic liposomes for gene and cell therapy. *J Biomed Biotechnol* 2012;2010:1-12
25. Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix B, Behr JP. A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. *Proc Natl Acad Sci U S A* 1995;92:7297-7301
26. Hunter AC. Molecular hurdles in polyfectin design and mechanistic background to polycation induced cytotoxicity. *Adv Drug Deliv Rev* 2006;58:1523-1531
27. Godbey WT, Wu KK, Mikos AG. Tracking the intracellular path of poly(ethylenimine)/DNA complexes for gene delivery. *Proc Natl Acad Sci U S A* 1999;96:5177-5181
28. Brandén LJ, Mohamed AJ, Smith CI. A peptide nucleic acid-nuclear localization signal fusion that mediates nuclear transport of DNA. *Nat Biotechnol* 1999;17:784-787
29. Ziemienowicz A, Görlich D, Lanka E, Hohn B, Rossi L. Import of DNA into mammalian nuclei by proteins originating from a plant pathogenic bacterium. *Proc Natl Acad Sci U S A* 1999;96:3729-3733
30. Subramanian A, Ranganathan P, Diamond SL. Nuclear targeting peptide scaffolds for lipofection of nondividing mammalian cells. *Nat Biotechnol* 1999;17:873-877
31. Cary LC, Goebel M, Corsaro BG, Wang HG, Rosen E, Fraser MJ. Transposon mutagenesis of baculoviruses: analysis of *Trichoplusia ni* transposon IFP2 insertions within the FP-locus of nuclear polyhedrosis viruses. *Virology* 1989;172:156-169
32. Boland MJ, Hazen JL, Nazor KL, Rodriguez AR, Gifford W, Martin G, Kupriyanov S, Baldwin KK. Adult mice generated from induced pluripotent stem cells. *Nature* 2009;461:91-94
33. Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, Ebina W, Mandal PK, Smith ZD, Meissner A, Daley GQ, Brack AS, Collins JJ, Cowan C, Schlaeger TM, Rossi DJ. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell* 2010;7:618-630
34. Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, Trauger S, Bien G, Yao S, Zhu Y, Siuzdak G, Schöler HR, Duan L, Ding S. Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell* 2009;4:381-384