We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 9

Induced Pluripotent Stem Cells for Clinical Use

Tomohisa Seki and Keiichi Fukuda

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62505

Abstract

Induced pluripotent stem cells (iPSCs) are expected to be a novel cell source for regenerative medicine. Although iPSCs represented a significant breakthrough, there were many initial obstacles for their clinical use such as exogenous sequence insertions, inefficient cell reprogramming, tumorigenic properties, and animal-derived culture components. However, much progress has been made in iPSC generation since their development. The first human trial of iPSC-derived cell transplantation was conducted in September 2014, in which iPSC-derived retinal pigment epithelial cells were transplanted to a patient with macular degeneration. Because multiple clinical trials using iPSCs are expected in the near future, preparation of guidelines for generating and selecting iPSC lines suitable for clinical application is a pressing issue.

For clinical use of iPSCs, many examinations for evaluating iPSC lines must be conducted before transplantation. Different combinations of reprogramming factors, gene derivation vehicles, and types of donor cells can affect the quality of iPSCs, and guidelines for selecting the most appropriate iPSC lines for clinical use are under development. Furthermore, development of time- and cost-effective selection methods is essential for expanding iPSC transplantation therapy. In this chapter, we review methods for preparing human iPSCs before clinical use and the issues that are important for defining standardization of clinical-grade iPSCs.

Keywords: induced pluripotent stem cells, regenerative medicine, transplantation therapy, standardization of stem cells, quality control of stem cells

1. Introduction

Induced pluripotent stem cells (iPSCs) are expected to be a novel cell source for regenerative therapy [1, 2]. Their capacity for self-proliferation and multilineage potential is promising for induction of regenerative cells without a natural capacity for self-renewal. In 2014, a ground-

breaking advance in iPSC research occurred when iPSC-derived sheets of retinal pigment epithelium were transplanted to a patient with age-related macular degeneration, the first report of iPSC treatment in humans [3]. Since then, explosive expansion clinical iPSC treatment of patients with otherwise intractable diseases has been expected. However, expanding the clinical use of iPSCs requires a well-defined quality standard. Generating clinical-grade iPSCs for regenerative treatments presents many challenges, and concerns over safe iPSC use must be solved promptly. For instance, ensuring that culture conditions are not exposed to risk of contamination by predictable or unpredictable agents requires a great deal of investment in terms of cost and equipment. In addition, the most clinically applicable method of generating and selecting a suitable iPSC line is under debate. Furthermore, although the construction of iPSC banks for allo-transplantation has progressed [4], generating sufficient qualified cell lines for clinical use requires several years to cover a large segment of the population. In this chapter, we discuss the current issues for expanding the clinical applications of human iPSCs.

2. Existing consensus guidelines for human pluripotent stem cells

In regard to the standardization of human pluripotent stem cells, a consensus for using embryonic stem cells (ESCs) was previously announced by the International Stem Cell Banking Initiative (ISCBI) contributors and the Ethics Working Party of the International Stem Cell Forum [5]. This consensus defined general principles for human ESC banking and described quality control processes for human ESC lines. Although many of these criteria can be applied to iPSCs, standardization of iPSC lines for clinical application is not fully established because there exist various iPSC generation methods and differences between iPSC lines. The tumorigenic and differentiation properties of iPSC lines are not identical, even for those generated by the same procedure [6]. Therefore, to establish iPSC quality standards for clinical use, determining which factors can affect iPSC quality and setting up requirements for clinical application of iPSCs are critical issues. This challenge intrinsically questions whether iPSCs can be equated with ESCs. To date, the existence of epigenetic differences between iPSCs and ESCs has been shown [7], although these differences do not negate the applicability of iPSCs.

Recently, the previous consensus on human pluripotent stem cells was revisited with consideration of iPSCs [8]. However, international standardization of iPSC generation techniques and quality verification is challenging. Many problems must be solved, including the scientific validity of new insight into iPSCs, to determine their applicability to consensus guidelines. In addition, these guidelines mainly target requirements for cell banking. In the case of iPSCs, there will be clinical research using autogenic iPSCs similar to the first case in RIKEN [3]. Therefore, the number of institutions in which autogenic iPSCs are generated could increase above the number of institutions for cell banking. Whether the institutional criteria for generating autogenic iPSCs should be equal to those for cell banking remains vague. These points should respectively be verified and adjusted based on scientific acceptability.

http://dx.doi.org/10.5772/62505

3. Development of the definition of "clinical-grade iPSCs"

When human iPSCs are applied for clinical use as a transplantation cell source, the requirements for iPSCs to satisfy clinical conditions must be validated in advance. Although most provisions for "clinical-grade" iPSCs are associated with "safety," this term encompasses many elements at each stage of iPSC application to regenerative medicine. However, the most suitable and the safest method for generating iPSCs for clinical use has not been defined because experience in treating patients with iPSC-derived regenerative cells remains limited. Therefore, to establish novel iPSC-based regenerative therapy, all factors that might affect safety must be presented and discussed in each case.

For clinical application of iPSCs, safety is mainly divided into two considerations. The first is that iPSCs must meet standards for general cell products. To establish iPSCs of clinical grade, cell culture protocols must avoid any risk of contamination with unpredictable pathogens and meet the standards for general cell products, such as good manufacturing practice (GMP) [5, 8]. Removing animal-derived products from the culture system is important for this purpose. Furthermore, iPSCs and iPSC-derived cells must not include pathogens such as harmful viruses or bacteria before clinical use. With respect to establishing cell culture protocols, accurate sample identification must be provided throughout iPSC generation and differentiation. Therefore, to meet the standards for general cell products, the establishment of extensive systems and equipment for culturing iPSCs is required.

The second aspect of safety is meeting the high quality standards for clinical applicability. However, whereas standards for general cell products have been defined, standards for high-quality iPSCs that meet applicability to clinical use remain vague. For example, methods for denying the possible tumorigenicity of iPSCs and their derivatives remain undefined. In addition, in the course of generating iPSCs, many steps affect iPSC quality. Worldwide standardization of each stage of iPSC generation is desirable, but there are numerous problems to be solved before achieving this goal.

4. Management of safety in each stage of iPSC generation

The first step for applying iPSCs application to clinical use is sampling somatic cells from donors. Although this step appears simple, it already includes safety considerations. When treatment with iPSCs is proposed, whether iPSCs are generated from the patient's own somatic cells or brought from a pool of allogeneic iPSCs such as the iPSC bank project [4] must be decided. Using allogeneic iPSCs requires co-treatment with an immune suppressor and can lead to a risk of malignant tumor and adverse effects. Generating autogenic iPSCs for each patient is ideal, but it requires a tremendous cost and time investment. Particularly, if the patient's condition demands expediency, autogenic iPSCs might not be suitable. Even if autogenic iPSCs are available, the appropriateness of applying quality standards for the allogenic iPSC bank to autogenic iPSCs has to be considered. In addition, the choice of somatic cell sources for iPSC generation is important. Naturally, invasive cell sampling is not prefera-

ble, but the type of original cells can affect iPSC features through the residual epigenetic status of the original cells [9–12]. Therefore, this first step already requires evaluation of the appropriate choice for each case.

The step following somatic cell sampling is somatic cell reprogramming. In this stage, the suitability of the combination of reprogramming factors and gene introduction vehicles must be verified. Although achievement of residual transgene-free iPSC lines was already established [13–19], the safest and most preferred type of gene vehicle and combination of reprogramming factors for clinical use are now in discussion. In addition, there are many reagent options for culturing human iPSCs, and reagent selection must be verified in advance.

The next stage of clinical therapy using iPSCs is the induction of targeted cells from iPSCs. iPSC lines do not exhibit identical points of differentiation [6, 12]. Although efficient induction of intended differentiated cells and selection of suitable cell lines are important, ensuring the safety of iPSC derivatives is a more important consideration. In particular, avoiding contamination of undifferentiated cells is essential for achieving clinical application. However, methods for detecting residual undifferentiated cells also remain undefined. Selection of appropriate iPSC lines is also important for avoiding tumorigenesis, which is known to differ among cell lines [20].

After obtaining targeted cells for treatment, the method by which these cells are transplanted is also associated with safety concerns. An appropriate transplantation protocol must be examined and established in advance. In addition, establishment of safety nets for post-treatment patients is also important.

Therefore, although the requirements for establishing a definitive standard for clinical-grade iPSCs are unresolved, many points that affect the safety of treatments must be recognized and appropriately verified before initiating treatment.

5. Advancement of reagents for iPSC generation and culture

As described above, meeting standards for general cell products is required for clinical use of iPSCs. A culture condition for human pluripotent stem cells was first established for maintaining human ESCs [21] and contained several animal-derived products such as mouse embryonic fibroblasts for feeder layers and fatal bovine serum in culture medium. This culture system was applied to human iPSCs, and it successfully maintained their pluripotency and self-proliferation [2]. Since then, toward realizing the clinical use of human iPSCs, the need for an established, chemically defined condition for human iPSCs has attracted attention.

To this end, many researchers have challenged the removal of animal-derived feeder cells from culture conditions. The initial condition for culturing human iPSCs contained mouse embryonic fibroblasts or immortalized mouse fibroblasts such as SNL cells [2]. Although auto-fibroblasts were applied and successfully served as alternative to animal-derived feeder cells for human iPSC generation [22], the availability of human auto-fibroblasts is quantitatively limited. Therefore, to apply human iPSCs to clinical use, replacing feeder layers with a chemically defined substitute is required.

Previously, gelatinous protein mixtures were applied to culturing human pluripotent stem cells. For example, human ESCs were successfully maintained with Matrigel and chemically defined medium [23]. Although Matrigel was applicable for maintenance of human pluripotent stem cells [24–26] and achieved feeder-free human iPSC generation [27, 28], this condition was not animal product-free because the matrix is derived from Engelbreth-Holm-Swarm mouse tumor [29] and contains many types of collagens, laminin, and proteoglycans. Therefore, the essential components for human iPSC culture have been investigated.

Other types of matrices, such as CellStart [30, 31] and synthetic polymers [32, 33], were tested and successfully used as feeder cell substitutes for the maintenance and generation of human pluripotent stem cells. In addition, recombinant cell adhesion proteins have received attention as a defined alternative for feeder cells. For example, vitronectin is a glycoprotein present in the extracellular matrix that mediates cell adhesion and was shown to be an alternative for feeder cells in human pluripotent stem cell culture [34]. Laminin, a component of the basal lamin, is another possible alternative to feeder cells in maintenance and generation of human iPSCs [35, 36]. These products allow removal of animal-derived feeder layers from human iPSC cultures and, therefore, are useful for establishing xeno-free culture conditions for human iPSCs.

The initial culture medium for iPSCs also contained animal products such as fatal bovine serum. There have been many subsequent reports of xeno-free media such as TeSR2 [37], NutriStem [38], Essential E8 [34], and StemFit [39] for human iPSC generation and maintaining. The combination of these matrices and media can achieve generation of human iPSCs under completely defined conditions, thus making iPSC generation in xeno-free conditions achievable.

6. Choice of iPSC donor cell sources

There are two considerations when choosing the types of donor cells. The first is whether allogenic iPSCs or autogenic iPSCs will be used. Applying autogenic iPSCs to each patient is ideal because this method is not expected to require co-treatment with an immune suppressor [40, 41]. The first case of therapy using iPSCs was performed using autogenic iPSCs [3] and was important for reaffirming the usefulness of iPSCs as a source of autogenic regenerative cells. Nevertheless, because of the immense amount of time and effort required to make autogenic iPSCs from each patient, allogenic iPSCs that are matched in human leukocyte antigen (HLA) type are an important option for establishing treatment with iPSC-derived cells [4], especially in cases that demand expedient treatment. However, covering an entire population with HLA-matched allogenic iPSCs is nearly impossible due to the high diversity of HLA genes [42]. Therefore, to achieve complete coverage of the population with iPSC banks is an important issue. In previous reports, hypoimmunogenic human pluripotent stem cells were successfully generated through genome editing [43–45]. Although these methods could complete the missing part of the iPSC bank, whether these genome-edited pluripotent cells are safe needs further validation. At this time, the imperfect coverage of iPSC banks has to be

recognized. When the time limit for generating iPSCs is not severe, autogenic iPSCs might become an important option. Therefore, whether standard guidelines for clinical-grade iPSCs can be defined equally for allogenic and autogenic iPSCs is in question. In a recent report, contaminated undifferentiated iPSC-derived cells readily grew teratomas in syngeneic conditions but not in allogenic conditions supported with an immune suppressor [46]. This difference of tumorigenesis between iPSC derivatives in autogenic and allogenic conditions could complicate definition of standards for clinical-grade iPSCs.

The other consideration about choosing types of donor cells is selection of the type of somatic cells for reprogramming. To date, there have been many efforts to minimize the invasiveness of sample acquisition. Previously, generating iPSCs from keratinocytes derived from plucked hair [47], fibroblasts derived from oral mucosa [48], and peripheral blood cells obtained by venipuncture [49–51] was established as less-invasive methods. However, the characteristics of iPSCs can be affected by the type of somatic cells used for their generation [10–12]. Whether residual epigenetic memory derived from original somatic cells is permissible in clinical-grade iPSCs must be considered. In addition, although blood cells are becoming the preferred material for iPSC generation, the best choice for generating iPSCs of high quality avoiding capture of somatic mutations and aberrant epigenetic memory remains undefined. These questions remain important issues toward standardization of iPSC quality.

7. Vehicles for gene delivery in iPSC generation

In generating iPSCs for clinical use, achieving residual transgene-free products is essential because of the possibly harmful effect of residual transgenes. Since the first report of human iPSC generation with retroviral gene introduction [2], there has been much technical progress in methods of gene introduction for iPSC generation. Currently, methods of generating transgene-free human iPSCs are established using adenovirus vectors [13], sendai virus vectors [14], transposons [16], RNA [18], recombinant protein [15, 17], or episomal vectors [19]. Even when non-viral methods such as episomal vectors are used, there is low incidence of genomic insertion of exogenous sequence. In the case of viral vectors, transposons, and episomal vectors, verification of vehicle elimination in iPSCs is necessary. Because methods for verifying the removal of these vehicles are not identical in each case, unionization and standardization of verification methods are difficult. An appropriate method must be established for each type of vehicle, or whole-genome sequencing to detect aberrant vehicle-derived sequence insertions might be essential for identifying residual transgene sequences in iPSCs.

8. Reprogramming factors and alternative molecules

In the first report of successful mouse somatic cell reprogramming with exogenous gene introduction, forced expression of *OCT3/4*, *SOX2*, *KLF4*, and *C-MYC* was introduced into mouse somatic cells [1]. Thereafter, although the resulting reprogramming efficiency was low,

http://dx.doi.org/10.5772/62505

C-MYC was shown to be dispensable for somatic cell reprogramming [52]. Another study showed that forced expression of *OCT3/4*, *SOX2*, *LIN28*, and *NANOG* also induced human somatic cell reprogramming [53]. To date, there have been many efforts to generate high-quality iPSCs, although the best combination of factors has not been established. To obtain safe iPSCs, alternative genes for *C-MYC* are needed because *C-MYC* is an oncogenic gene [54], and residual expression of *C-MYC* in iPSCs led to tumorigenesis [52]. TBX3 [55], L-MYC [56], and GLIS1 [57] were reported as alternatives to *C-MYC* with improved chimerism, germline contribution, or prognosis of iPSC-derived clone mice. Although replicating the chimerism experiments using human iPSCs is difficult because of ethical problems, these alternatives to *C-MYC* are expected to provide human iPSCs of high quality.

At the other extreme, there have been many efforts to generate iPSCs using chemical compounds. Although progress in developing gene vehicles enabled generation of residual transgene-free iPSCs, the ultimate goal is to generate iPSCs without gene introduction. Many previous reports demonstrated improved reprogramming efficiency with small molecules, with some specific small molecules serving as a substitute for reprogramming factors [58]. Finally, in mice, a combination of small molecules completely reprogrammed somatic cells into pluripotent states without forced expression of exogenous genes [59, 60]. Although these chemically generated iPSCs require further verification in terms of quality such as residual epigenetic modification of somatic cells, they have the potential to become mainstream for iPSC-associated researches.

9. Quality control of iPSC lines

The quality of mouse iPSCs has been mainly evaluated through chimerism experiments [61]. Germline contribution of iPSCs and induction of iPSC-derived mice have been the ultimate verifications of pluripotency. Previous reports of iPSC generation with TBX3 [55], L-MYC [56], or GLIS1 [57] also evaluated the quality of iPSCs through mouse chimera formation and germline contribution. However, these experiments are not applicable to human iPSCs because of ethical concerns. In addition, although an in vivo teratoma formation assay has also been used to establish the differentiation capacity of iPSCs, quantifying teratoma formation is rather difficult in contrast to in vitro differentiation because the amount of time to teratoma formation and pathological interpretation are needed. Therefore, the quality of human iPSCs has been evaluated with an in vitro differentiation assay.

For example, the in vitro differentiation assay of human iPSCs revealed that differentiation was affected by donor cell types [10–12]. This phenomenon is termed "epigenetic memory" and, interestingly, does not arise from somatic cell reprogramming with nuclear transfer. Although epigenetic memory decreases with increasing culture time [12], residual epigenetic modification of iPSC origin cells must be considered when iPSCs are applied to clinical use. In addition, the gene expression of iPSCs showing a tumorigenic tendency after neural differentiation was analyzed [20], revealing that activated expression of genes containing specific LTR7 sequences in iPSCs was statistically associated with tumorigenesis. Such

predictive markers for the quality of human iPSCs are important for rapid selection of cell lines suitable for clinical use.

Recent progress in next-generation sequencing (NGS) techniques has provided platforms for exhaustive analysis of iPSC RNA, genome, and epigenome. This type of analysis can detect chromosomal aberrations in human iPSCs as sequence abnormalities [62]. Although whether somatic cell reprogramming itself can lead to genomic abnormalities in iPSCs is in discussion, long-term culture of pluripotent stem cells is known to lead to genomic abnormalities [63]. Because mutations in protein-coding regions of the genome could trigger tumorigenesis of iPSCs, analysis of iPSCs with NGS can assume a large role in evaluating iPSC quality and selecting iPSCs suitable for clinical use.

As presented above, evaluation of human iPSC quality has been performed without chimera assays. Whether all of perceptions in mouse iPSC experiments are directly applicable to human iPSCs remains a matter of debate. Therefore, investigating the chimeric contribution capacity of human iPSCs has an ultimate importance in quantifying the pluripotency of human iPSCs. In this regard, recent analysis indicated the possibility of transcending boundaries between human iPSCs and chimera formation assays. Human iPSCs and ESCs have features similar to mouse epiblast stem cells, which are in an advanced differentiation state compared to mouse ESCs [64]. Common human iPSCs and mouse epiblast stem cells are thought to be in a "primed state" distinct from the "naïve state" of mouse ESCs. Pluripotent stem cells in a primed state have difficulty in contributing chimeras in preimplantation embryos [65]. In addition, generating chimeras of human and mouse cells is ethically problematic. Therefore, methods for evaluating the quality of human iPSCs have not been standardized. However, a recent report showed that human iPSCs could contribute to chimeras in stage-matched post-implantation mouse embryos [66]. Although the observation period of chimeric embryos was limited, chimera formation experiments with human iPSCs and stage-matching post-implantation mouse embryos might represent a novel assay for evaluating the quality of human iPSCs.

10. Quality control of iPSC-derived products

iPSCs are used as a cell source for inducing intended types of differentiated cells and are not transplanted to patients directly. Therefore, as with quality control of iPSCs, quality control of iPSC-derived products is important for ensuring the safety of clinical application of iPSCs and contains two important considerations for achieving safety.

The first is purification of intended cells from a mixture of differentiated cells. Even a small contamination of undifferentiated cells in the final product could lead to teratoma development after transplantation [67]. Therefore, appropriate methods for purification are required to ensure safety. Purification of products derived from pluripotent stem cells has been previously attempted using a surface marker of pluripotent stem cells to remove undifferentiated cells [68, 69] or cell sorting targeting surface markers specific to intended cells [70, 71]. However, fluorescence-activated cell sorting with cell surface markers is difficult to apply to mass culture systems because of the large time investment required. To achieve applicability

to mass culture systems, purification with medium conditions [72, 73] or with reagents with specific cytotoxic effects against undifferentiated cells [74, 75] was developed. These techniques are expected to be useful for achieving safe iPSC-derived products.

To validate the quality of products derived from iPSCs, detection of residual undifferentiated cells in the product is another essential technique for avoiding tumorigenesis after transplantation. If the methods described above for purification achieve high accuracy, methods for evaluating the elimination of undifferentiated cells and assuring safety are required. However, current validation methods for detecting residual undifferentiated cells in final products from iPSCs are limited. Examining expression of pluripotent markers in products from iPSCs with qRT-PCR [76, 77] and detecting specific glycoproteins in the cell culture supernatants [78] were reported as useful methods for evaluating elimination of undifferentiated cells. To ultimately demonstrate safety, the absence of tumorigenesis in in vivo transplantation assays is required, but the appropriate observation period and numerous transplanted cells remain evasive. In addition, whether xeno-transplantation experiments truly replicate transplantation of human cases needs further validation.

11. Building safety nets for post-treatment patients

One of the most important issues for clinical iPSC application is the establishment of safety nets for post-treatment patients. If tumorigenic cell contaminates the final iPSC-derived product and might be transplanted into patients, measures to avoid health hazard to patients must be established. Although surgical resection of iPSC-derived tumors is one conceivable method, there will be cases that cannot be managed through surgery due to the invasiveness of the operation.

Introducing a suicide system into human iPSCs before transplantation is another useful approach for ensuring safe clinical application of iPSCs. When iPSC-derived tumors occur in patients, ablation of iPSC-derived cells by switching the suicide system "on" can prevent invasive surgery in high-risk patients. For example, herpes simplex virus thymidine kinase (HSV-TK) phosphorylates ganciclovir (GCV) and leads to cytotoxicity in the presence of GCV. HSV-TK has been widely used as "suicide gene" in human ESC experiments [79]. The combination of HSV-TK and GCV was also tested in an in vivo mouse model with mouse iPSCs [80, 81]. Another possibility is the combination of inducible caspase-9 and a chemical inducer of dimerization, which was shown to work as suicide system in human iPSC derivatives [82]. Whereas the HSV-TK suicide system is cell cycle dependent, inducible caspase-9 achieves cell cycle-independent ablation of target cells. Although these systems can become an important option for treatment of iPSC-derived tumors, modified genomic introduction methods of suicide genes are required for clinical use. Because random exogenous introduction using lentiviral and retroviral vectors could break functional gene sequences in iPSCs, validation of target sites for suicide gene insertion and targeted genome editing in iPSCs is required for clinical application.

This type of strategy has a disadvantage in that all iPSC-derived cells are diminished with the suicide system. When iPSC-derived tumors occur, diminishing only tumor cells while retaining the useful cells in the engrafted treatment is ideal. With this in mind, some reports showed selective suicide systems in which the suicide gene is inserted under control of a promoter of pluripotent markers [80]. However, in this area of research, how selective removal of tumor cells should be ensured remains to be solved.

Currently, in contrast to research ensuring the safety of iPSCs and iPSC-derivatives, research establishing methods to manage cases in which iPSC-derived tumors occur in post-treatment patients is less common. To ensure safe clinical application of iPSCs, countermeasures for every possible contingency after treatment using iPSCs must be prepared in advance. Thus, this type of research is of considerable importance in the area of regenerative medicine.

12. Conclusion

iPSCs are expected to serve as a novel cell source for regenerative medicine, although there are many points that require verification before expanding their application to broad clinical uses. Standardization of iPSC quality is required, but current verification and validation procedures are not perfect. This incompleteness must be widely recognized. To establish safe iPSC use in regenerative therapy, appropriate improvements of these issues and defined guidelines for iPSCs are expected.

Author details

Tomohisa Seki[1,2*] and Keiichi Fukuda[2]

*Address all correspondence to: seki@a6.keio.jp

1 Department of Emergency and Critical Care Medicine, Keio University School of Medicine, Shinjuku-ku Tokyo, Japan

2 Department of Cardiology, Keio University School of Medicine, Shinjuku-ku Tokyo, Japan

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] 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.

[3] 365 days: Nature's 10. Nature 2014;516(7531):311–9.

[4] Cyranoski D. Stem-cell pioneer banks on future therapies. Nature 2012;488(7410):139.

[5] The International Stem Cell Banking Initiative. Consensus guidance for banking and supply of human embryonic stem cell lines for research purposes. Stem Cell Review 2009;5(4):301–14.

[6] Kajiwara M, Aoi T, Okita K, Takahashi R, Inoue H, Takayama N, et al. Donor-dependent variations in hepatic differentiation from human-induced pluripotent stem cells. Proceedings of the National Academy of Sciences of the United States of America 2012;109(31):12538–43.

[7] Ma H, Morey R, O'Neil RC, He Y, Daughtry B, Schultz MD, et al. Abnormalities in human pluripotent cells due to reprogramming mechanisms. Nature 2014;511(7508): 177–83.

[8] Andrews PW, Baker D, Benvinisty N, Miranda B, Bruce K, Brustle O, et al. Points to consider in the development of seed stocks of pluripotent stem cells for clinical applications: International Stem Cell Banking Initiative (ISCBI). Regenerative Medicine 2015;10(2 Suppl):1–44.

[9] Miura K, Okada Y, Aoi T, Okada A, Takahashi K, Okita K, et al. Variation in the safety of induced pluripotent stem cell lines. Nature Biotechnology 2009;27(8):743–5.

[10] Kim K, Doi A, Wen B, Ng K, Zhao R, Cahan P, et al. Epigenetic memory in induced pluripotent stem cells. Nature. 2010;467(7313):285–90.

[11] Polo JM, Liu S, Figueroa ME, Kulalert W, Eminli S, Tan KY, et al. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. Nature Biotechnology 2010;28(8):848–55.

[12] Bar-Nur O, Russ HA, Efrat S, Benvenisty N. Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. Cell Stem Cell 2011;9(1):17–23.

[13] Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. Science (New York, NY) 2008;322(5903):945–9.

[14] Fusaki N, Ban H, Nishiyama A, Saeki K, Hasegawa M. 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. Proceedings of the Japan Academy. Series B, Physical and Biological Sciences 2009;85(8):348–62.

[15] 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.

[16] Woltjen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, Hamalainen R, et al. PiggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 2009;458(7239):766–70.

[17] Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, et al. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 2009;4(5):381–4.

[18] 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.

[19] Okita K, Matsumura Y, Sato Y, Okada A, Morizane A, Okamoto S, et al. A more efficient method to generate integration-free human iPS cells. Nature Methods 2011;8(5):409–12.

[20] Koyanagi-Aoi M, Ohnuki M, Takahashi K, Okita K, Noma H, Sawamura Y, et al. Differentiation-defective phenotypes revealed by large-scale analyses of human pluripotent stem cells. Proceedings of the National Academy of Sciences of the United States of America 2013;110(51):20569–74.

[21] Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science (New York, NY) 1998;282(5391):1145–7.

[22] Takahashi K, Narita M, Yokura M, Ichisaka T, Yamanaka S. Human induced pluripotent stem cells on autologous feeders. PLoS One 2009;4(12):e8067.

[23] Ludwig TE, Levenstein ME, Jones JM, Berggren WT, Mitchen ER, Frane JL, et al. Derivation of human embryonic stem cells in defined conditions. Nature Biotechnology 2006;24(2):185–7.

[24] Xu C, Inokuma MS, Denham J, Golds K, Kundu P, Gold JD, et al. Feeder-free growth of undifferentiated human embryonic stem cells. Nature Biotechnology 2001;19(10):971–4.

[25] Totonchi M, Taei A, Seifinejad A, Tabebordbar M, Rassouli H, Farrokhi A, et al. Feeder- and serum-free establishment and expansion of human induced pluripotent stem cells. International Journal of Developmental Biology 2010;54(5):877–86.

[26] Stover AE, Schwartz PH. Adaptation of human pluripotent stem cells to feeder-free conditions in chemically defined medium with enzymatic single-cell passaging. Methods in Molecular Biology (Clifton, NJ) 2011;767:137–46.

[27] Lai WH, Ho JC, Lee YK, Ng KM, Au KW, Chan YC, et al. ROCK inhibition facilitates the generation of human-induced pluripotent stem cells in a defined, feeder-, and serum-free system. Cell Reprogram 2010;12(6):641–53.

[28] Kishino Y, Seki T, Fujita J, Yuasa S, Tohyama S, Kunitomi A, et al. Derivation of transgene-free human induced pluripotent stem cells from human peripheral T cells in defined culture conditions. PLoS One 2014;9(5):e97397.

[29] Emonard H, Grimaud JA, Nusgens B, Lapiere CM, Foidart JM. Reconstituted basement-membrane matrix modulates fibroblast activities in vitro. Journal of Cellular Physiology 1987;133(1):95–102.

[30] Ausubel LJ, Lopez PM, Couture LA. GMP scale-up and banking of pluripotent stem cells for cellular therapy applications. Methods in Molecular Biology (Clifton, NJ) 2011;767:147–59.

[31] Bergstrom R, Strom S, Holm F, Feki A, Hovatta O. Xeno-free culture of human pluripotent stem cells. Methods in Molecular Biology (Clifton, NJ) 2011;767:125–36.

[32] Mei Y, Saha K, Bogatyrev SR, Yang J, Hook AL, Kalcioglu ZI, et al. Combinatorial development of biomaterials for clonal growth of human pluripotent stem cells. Nature Materials 2010;9(9):768–78.

[33] Lu HF, Narayanan K, Lim SX, Gao S, Leong MF, Wan AC. A 3D microfibrous scaffold for long-term human pluripotent stem cell self-renewal under chemically defined conditions. Biomaterials 2012;33(8):2419–30.

[34] Chen G, Gulbranson DR, Hou Z, Bolin JM, Ruotti V, Probasco MD, et al. Chemically defined conditions for human iPSC derivation and culture. Nature Methods 2011;8(5):424–9.

[35] Rodin S, Domogatskaya A, Strom S, Hansson EM, Chien KR, Inzunza J, et al. Long-term self-renewal of human pluripotent stem cells on human recombinant laminin-511. Nature Biotechnology 2010;28(6):611–5.

[36] Miyazaki T, Futaki S, Suemori H, Taniguchi Y, Yamada M, Kawasaki M, et al. Laminin E8 fragments support efficient adhesion and expansion of dissociated human pluripotent stem cells. Nature Communications 2012;3:1236.

[37] Meng G, Liu S, Rancourt DE. Synergistic effect of medium, matrix, and exogenous factors on the adhesion and growth of human pluripotent stem cells under defined, xeno-free conditions. Stem Cells and Development 2012;21(11):2036–48.

[38] Sugii S, Kida Y, Kawamura T, Suzuki J, Vassena R, Yin YQ, 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 2010;107(8):3558–63.

[39] Nakagawa M, Taniguchi Y, Senda S, Takizawa N, Ichisaka T, Asano K, et al. A novel efficient feeder-free culture system for the derivation of human induced pluripotent stem cells. Scientific Reports 2014;4:3594.

[40] Araki R, Uda M, Hoki Y, Sunayama M, Nakamura M, Ando S, et al. Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. Nature 2013;494(7435):100–4.

[41] Guha P, Morgan JW, Mostoslavsky G, Rodrigues NP, Boyd AS. Lack of immune response to differentiated cells derived from syngeneic induced pluripotent stem cells. Cell Stem Cell 2013;12(4):407–12.

[42] Zimmermann A, Preynat-Seauve O, Tiercy JM, Krause KH, Villard J. Haplotype-based banking of human pluripotent stem cells for transplantation: Potential and limitations. Stem Cells and Development 2012;21(13):2364–73.

[43] Riolobos L, Hirata RK, Turtle CJ, Wang PR, Gornalusse GG, Zavajlevski M, et al. HLA engineering of human pluripotent stem cells. Molecular Therapy: The Journal of the American Society of Gene Therapy 2013;21(6):1232–41.

[44] Rong Z, Wang M, Hu Z, Stradner M, Zhu S, Kong H, et al. An effective approach to prevent immune rejection of human ESC-derived allografts. Cell Stem Cell 2014;14(1): 121–30.

[45] Wang D, Quan Y, Yan Q, Morales JE, Wetsel RA. Targeted disruption of the beta2-microglobulin gene minimizes the immunogenicity of human embryonic stem cells. Stem Cells Translational Medicine 2015;4(10):1234–45.

[46] Kawamura A, Miyagawa S, Fukushima S, Kawamura T, Kashiyama N, Ito E, et al. Teratocarcinomas arising from allogeneic induced pluripotent stem cell-derived cardiac tissue constructs provoked host immune rejection in mice. Scientific Reports 2016;6:19464.

[47] Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, Gonzalez F, et al. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nature Biotechnology 2008;26(11):1276–84.

[48] Miyoshi K, Tsuji D, Kudoh K, Satomura K, Muto T, Itoh K, et al. Generation of human induced pluripotent stem cells from oral mucosa. Journal of Bioscience and Bioengineering 2010;110(3):345–50.

[49] Loh YH, Hartung O, Li H, Guo C, Sahalie JM, Manos PD, et al. Reprogramming of T cells from human peripheral blood. Cell Stem Cell 2010;7(1):15–9.

[50] Seki T, Yuasa S, Oda M, Egashira T, Yae K, Kusumoto D, et al. Generation of induced pluripotent stem cells from human terminally differentiated circulating T cells. Cell Stem Cell 2010;7(1):11–4.

[51] Staerk J, Dawlaty MM, Gao Q, Maetzel D, Hanna J, Sommer CA, et al. Reprogramming of human peripheral blood cells to induced pluripotent stem cells. Cell Stem Cell 2010;7(1):20–4.

[52] Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, et al. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nature Biotechnology 2008;26(1):101–6.

[53] 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 (New York, NY). 2007;318(5858):1917–20.

[54] Dang CV. MYC on the path to cancer. Cell 2012;149(1):22–35.

[55] Han J, Yuan P, Yang H, Zhang J, Soh BS, Li P, et al. Tbx3 improves the germ-line competency of induced pluripotent stem cells. Nature 2010;463(7284):1096–100.

[56] Nakagawa M, Takizawa N, Narita M, Ichisaka T, Yamanaka S. Promotion of direct reprogramming by transformation-deficient Myc. Proceedings of the National Academy of Sciences of the United States of America 2010;107(32):14152–7.

[57] Maekawa M, Yamaguchi K, Nakamura T, Shibukawa R, Kodanaka I, Ichisaka T, et al. Direct reprogramming of somatic cells is promoted by maternal transcription factor Glis1. Nature 2011;474(7350):225–9.

[58] Lin T, Wu S. Reprogramming with small molecules instead of exogenous transcription factors. Stem Cells International 2015;2015:794632.

[59] 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 (New York, NY) 2013;341(6146):651–4.

[60] Zhao Y, Zhao T, Guan J, Zhang X, Fuv Y, Ye J, et al. A XEN-like state bridges somatic cells to pluripotency during chemical reprogramming. Cell 2015;163(7):1678–91.

[61] Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature 2007;448(7151):313–7.

[62] Gore A, Li Z, Fung HL, Young JE, Agarwal S, Antosiewicz-Bourget J, et al. Somatic coding mutations in human induced pluripotent stem cells. Nature 2011;471(7336):63–7.

[63] Baker DE, Harrison NJ, Maltby E, Smith K, Moore HD, Shaw PJ, et al. Adaptation to culture of human embryonic stem cells and oncogenesis in vivo. Nature Biotechnology 2007;25(2):207–15.

[64] Mascetti VL, Pedersen RA. Naivete of the human pluripotent stem cell. Nature Biotechnology 2014;32(1):68–70.

[65] Masaki H, Kato-Itoh M, Umino A, Sato H, Hamanaka S, Kobayashi T, et al. Interspecific in vitro assay for the chimera-forming ability of human pluripotent stem cells. Development (Cambridge, England) 2015;142(18):3222–30.

[66] Mascetti VL, Pedersen RA. Human-mouse chimerism validates human stem cell pluripotency. Cell Stem Cell 2016;18(1):67–72.

[67] Blum B, Benvenisty N. The tumorigenicity of human embryonic stem cells. Advances in Cancer Research 2008;100:133–58.

[68] Tang C, Lee AS, Volkmer JP, Sahoo D, Nag D, Mosley AR, et al. An antibody against SSEA-5 glycan on human pluripotent stem cells enables removal of teratoma-forming cells. Nature Biotechnology 2011;29(9):829–34.

[69] Ben-David U, Nudel N, Benvenisty N. Immunologic and chemical targeting of the tight-junction protein Claudin-6 eliminates tumorigenic human pluripotent stem cells. Nature Communications 2013;4:1992.

[70] Dubois NC, Craft AM, Sharma P, Elliott DA, Stanley EG, Elefanty AG, et al. SIRPA is a specific cell-surface marker for isolating cardiomyocytes derived from human pluripotent stem cells. Nature Biotechnology 2011;29(11):1011–8.

[71] Uosaki H, Fukushima H, Takeuchi A, Matsuoka S, Nakatsuji N, Yamanaka S, et al. Efficient and scalable purification of cardiomyocytes from human embryonic and induced pluripotent stem cells by VCAM1 surface expression. PLoS One 2011;6(8):e23657.

[72] Tohyama S, Hattori F, Sano M, Hishiki T, Nagahata Y, Matsuura T, et al. Distinct metabolic flow enables large-scale purification of mouse and human pluripotent stem cell-derived cardiomyocytes. Cell Stem Cell 2013;12(1):127–37.

[73] Shiraki N, Shiraki Y, Tsuyama T, Obata F, Miura M, Nagae G, et al. Methionine metabolism regulates maintenance and differentiation of human pluripotent stem cells. Cell Metabolism 2014;19(5):780–94.

[74] Matsumoto S, Nakao H, Kawabe K, Nonaka M, Toyoda H, Takishima Y, et al. A cytotoxic antibody recognizing lacto-N-fucopentaose I (LNFP I) on human induced pluripotent stem (hiPS) cells. Journal of Biological Chemistry 2015;290(33):20071–85.

[75] Tateno H, Onuma Y, Ito Y, Minoshima F, Saito S, Shimizu M, et al. Elimination of tumorigenic human pluripotent stem cells by a recombinant lectin-toxin fusion protein. Stem Cell Reports 2015;4(5):811–20.

[76] Kuroda T, Yasuda S, Kusakawa S, Hirata N, Kanda Y, Suzuki K, et al. Highly sensitive in vitro methods for detection of residual undifferentiated cells in retinal pigment epithelial cells derived from human iPS cells. PLoS One 2012;7(5):e37342.

[77] Kuroda T, Yasuda S, Sato Y. In vitro detection of residual undifferentiated cells in retinal pigment epithelial cells derived from human induced pluripotent stem cells. Methods in Molecular Biology (Clifton, NJ) 2014;1210:183–92.

[78] Tateno H, Onuma Y, Ito Y, Hiemori K, Aiki Y, Shimizu M, et al. A medium hyperglycosylated podocalyxin enables noninvasive and quantitative detection of tumorigenic human pluripotent stem cells. Scientific Reports 2014;4:4069.

[79] Schuldiner M, Itskovitz-Eldor J, Benvenisty N. Selective ablation of human embryonic stem cells expressing a "suicide" gene. Stem Cells (Dayton, Ohio) 2003;21(3):257–65.

[80] Chen F, Cai B, Gao Y, Yuan X, Cheng F, Wang T, et al. Suicide gene-mediated ablation of tumor-initiating mouse pluripotent stem cells. Biomaterials 2013;34(6):1701–11.

[81] Lim TT, Geisen C, Hesse M, Fleischmann BK, Zimmermann K, Pfeifer A. Lentiviral vector mediated thymidine kinase expression in pluripotent stem cells enables removal of tumorigenic cells. PLoS One 2013;8(7):e70543.

[82] Ando M, Nishimura T, Yamazaki S, Yamaguchi T, Kawana-Tachikawa A, Hayama T, et al. A safeguard system for induced pluripotent stem cell-derived rejuvenated T cell therapy. Stem Cell Reports 2015;5(4):597–608.

IntechOpen