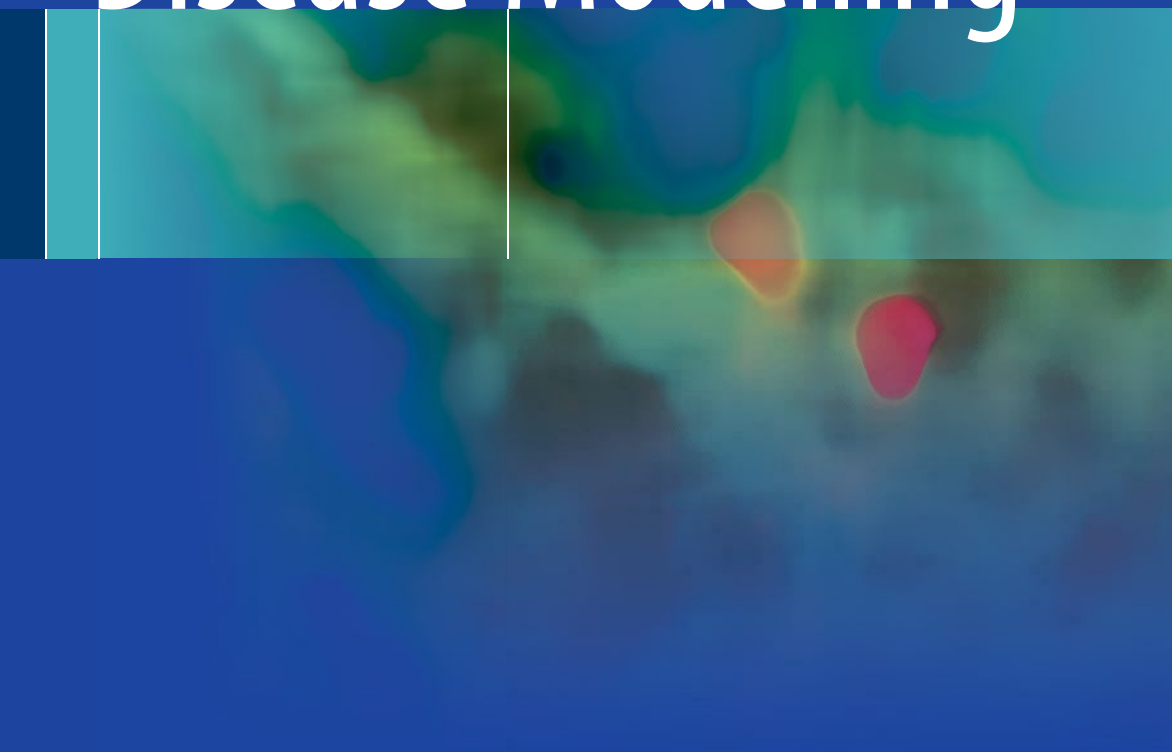
Keiichi Fukuda *Editor*

Human iPS Cells in Disease Modelling



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Preface

Recent advances in stem cell biology have enabled us to examine the regeneration of various tissues and organs. One of the biggest advances in this field is the induced pluripotent stem cell (iPS cell), which was developed by Shinya Yamanaka in 2006. The iPS cell was a tailor-made multipotent stem cell, and was generated by transfection of the combination of several embryonic stem (ES) cell-specific transcription factors such as Oct3/4, Sox2, and Klf4. It has pluripotency, and can differentiate into various types of cells such as ES cells. Because the iPS cells maintained all the genome information including HLA, it cannot be immune-rejected when its derived cells are transplanted to the host. In 2014, the first clinical trial was performed by Masayo Takahashi in patients with senile macular degeneration using autologous iPS cell-derived retinal pigmented cells. Other clinical trials are now being conducted in diverse ways including the cornea, Parkinson's Disease, spinal cord injury, platelet production, and severe congestive heart failure. Realization of these projects has been eagerly awaited by patients with severe intractable diseases.

It was also expected that iPS cells could be used in another field: disease modelling. Hereditary diseases are caused by genome mutations, but their clinical phenotypes, severity, onset, and treatment show wide variation. Mouse models of human disease have been generated by gene targeting and transgenic animals during the past 25 years. Nevertheless, these animal models cannot always help us to simulate human disease and screening of drugs. Genes and proteins are different between mice and humans. Because human tissues and cells were not usually available in the in vitro experiments except for small amounts of tissue obtained by biopsy or autopsy, this became a big hurdle for in vitro phenotype analysis and drug development. Moreover, disease modelling of human cells was not available in a routine clinical and experimental setting. Generation of patient-derived iPS cells and induction of in vitro differentiation into the targeted cells and tissues greatly changed the situation. Use of iPS cells for the investigation of disease modelling and drug screening is certain to change the future direction of research and industry.

In this book, we asked the top scientists in the field to write about human iPS cells for disease modelling. This will greatly help readers to understand what an iPS cell is, how to make iPS cells from blood cells, how to apply these techniques to approaching a pathophysiological analysis, and how to perform drug development

for patients with hereditary diseases. I strongly hope that readers can easily understand this field and will attempt disease modelling and tailor-made drug development for patients around the world.

Tokyo, Japan Keiichi Fukuda

Contents

Recent Improvements and Emerging Issues in iPSC Generation for the Modeling of Disease

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Tomohisa Seki, Shinsuke Yuasa, and Keiichi Fukuda

Abstract

Recently, induced pluripotent stem cells (iPSCs) have attracted attention as a novel tool for the modeling of disease because of their potential to reveal new insights that have not been elucidated using animal models. Since iPSC generation was first reported, there have been many efforts to improve the method of generating iPSCs for clinical applications. To date, many methods for iPSC generation have been reported. Each has advantages and disadvantages for the modeling of disease, and thus the most appropriate method differs depending on the intended use of the iPSCs. Additionally, as the study of disease modeling with human iPSCs has progressed, the need to remove uncertainties due to variations in iPSCs cell lines has increasingly focused researchers' attention on attaining experimental accuracy. Recognition of these uncertainties is important for the advancement of disease modeling studies with iPSCs.

Keywords

Induced pluripotent stem cells • Disease modeling • Cell reprogramming

1.1 Introduction

Since the reprogramming of somatic cells by forced expression of reprogramming factors was first reported (Takahashi and Yamanaka 2006), this technique has attracted attention as a novel tool for regenerative therapy and disease research. The cells obtained using this approach have been named induced pluripotent stem

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cells (iPSCs). The first method for generating human iPSCs was based on retrovirally mediated introduction of genes into primary cultures of fibroblasts (Takahashi et al. 2007). Since then, many efforts have aimed at improving the generation of iPSCs for clinical applications. However, the best method for generating iPSCs is a topic for discussion. Each method has advantages and disadvantages, and the most preferable choice should be based on the intended use of the iPSCs.

The reason for generating iPSCs falls into one of two major classifications. One is for transplantation therapy (Garber 2013) and the other is for disease research. iPSCs generated from patients are not only a source of cells for transplantation but are also a model of human disease. In vitro modeling of disease with a patient's own cells can possibly lead to novel insights that would be unattainable using an animal model.

To date, there have been many efforts to improve methods for generating iPSCs for clinical applications. For example, establishing xeno-free culture conditions and selecting non-tumorigenic iPSC lines are becoming more important for the use of iPSCs in transplantation therapy (Lee et al. 2013). It must be emphasized that generation of iPSCs for disease modeling is different to that of cells for future transplantation therapy. Establishing noninvasive cell sampling methods from patients for generating iPSCs (Yamanaka 2010) and avoiding host genome anomalies in the iPSCs are particularly important points to consider for generating iPSCs for disease modeling. Additionally, it is possible that iPSC lines do not always have the same characteristics, even when generated from the same donor (Kajiwara et al. 2012). Therefore, to understand disease modeling using iPSCs, it is important to focus on the features of iPSC generation methods and the quality of iPSC lines.

1.2 Combination of Transgenes for Somatic Cell Reprogramming

In the first report of iPSC generation, forced expression of *OCT3/4*, *SOX2*, *KLF4*, and *C-MYC* was used for successful somatic reprogramming (Takahashi and Yamanaka 2006). Since then, the combination of reprogramming factors required to generate iPSCs has been one of the most hotly debated issues in iPSC research. In many situations, the quality of iPSCs has been seen as an important condition for clinical use. For instance, *C-MYC* is an oncogenic gene (Dang 2012). Following the first report of iPSC generation, *C-MYC* was shown to be dispensable for iPSC generation in the mouse, although the reprogramming efficiency was dramatically lowered with the combination of only three factors: *OCT3/4*, *SOX2*, and *KLF4* (Nakagawa et al. 2008). Subsequently, *TBX3* (Han et al. 2010), *L-MYC* (Nakagawa et al. 2010), or *Glis1* (Maekawa et al. 2011) were shown to serve as a substitute for *C-MYC* and have a salutary effect on the efficiency in reprogramming somatic cells or germ line transmission of generated iPSCs. But, of course, validation of iPSC quality such as by the efficiency of germ line transmission is confined to experiments with nonhuman iPSCs. Therefore, the combination of reprogramming

factors appropriate for generating human iPSCs for modeling human disease has been a topic of discussion.

For generating iPSCs from patients, the efficiency of cell reprogramming has to be considered important because the chances of sampling a patient's cells or tissues are limited. Additionally, the amount of available tissue for generating a patient's iPSCs will be also limited. Therefore, establishing a stable system for generating human iPSCs forms the basis of experiments that model disease with iPSCs.

To date, many pathways have been recognized as targets for improving reprogramming efficiency. In particular, suppression of the p53-p21 pathway of tumor suppressor signals (Green and Kroemer 2009) enhances the efficiency of cell reprogramming (Hong et al. 2009; Kawamura et al. 2009; Li et al. 2009; Marion et al. 2009; Utikal et al. 2009). In fact, the short hairpin RNA (shRNA) of *p53* is included in the set of reprogramming factors used in the protocols provided by the Center for iPS Cell Research and Application (CiRA) to knock down the expression of *p53* and maintain the efficiency of somatic cell reprogramming (Okita et al. 2013). For establishing stable and efficient techniques for generating human iPSCs, the addition of factors that influence desired pathways and improve cell reprogramming efficiency is of considerable use.

1.3 Residual Transgene-Free Methods of Generating iPSCs

Initially, iPSCs were generated using a combination of fibroblast cultures and retroviral gene introduction (Takahashi et al. 2007; Takahashi and Yamanaka 2006). Subsequently, tumorigenic risk derived from reactivation of transgenes was reported (Okita et al. 2007), although transgenes that were introduced with retroviral vectors were silenced in the pluripotent state (Hawley et al. 1994; Stewart et al. 1982). Additionally, besides the influence of residual transgene expression, genomic insertion in itself has the potential to disrupt gene function and change gene expression in iPSCs. Interestingly, introduction of green fluorescent protein alone using lentivirus at an extremely high multiplicity of infection led to somatic cell reprogramming (Kane et al. 2010). This phenomenon is thought to be caused by multiple insertions of vector DNA into the genome and is also thought to be possible evidence for a harmful effect of insertional vectors. In cases of disease modeling with patient's iPSCs, factors that possibly affect the phenotype of patient-derived cells should be removed by all means. Therefore, gene introduction methods accompanied by genomic insertion are unfavorable for generating iPSCs, not only for transplantation therapy but also for disease modeling.

To avoid genomic insertion of transgenes, many methods have been established. Adenovirus (Stadtfeld et al. 2008), *Sendai virus* (Fusaki et al. 2009), transposons (Woltjen et al. 2009), RNA (Warren et al. 2010), recombinant protein (Kim et al. 2009; Zhou et al. 2009), and episomal vectors (Okita et al. 2011) are successful methods for introducing reprogramming factors and generating transgene-free iPSCs. However, these methods need additional checks to confirm the disappearance of