Review

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Modeling of Human Genetic Diseases Via Cellular Reprogramming

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The generation of induced pluripotent stem cells (iPSCs) derived from patients' somatic cells provides a new paradigm for studying human genetic diseases. Human iPSCs which have similar properties of human embryonic stem cells (hESCs) provide a powerful platform to recapitulate the disease-specific cell types by using various differentiation techniques. This promising technology has being realized the possibility to explore pathophysiology of many human genetic diseases at the molecular and cellular levels. Furthermore, disease-specific human iPSCs can also be used for patient-based drug screening and new drug discovery at the stage of the pre-clinical test *in vitro*. In this review, we summarized the concept and history of cellular reprogramming or iPSC generation and highlight recent progresses for disease modeling using patient-specific iPSCs.

Key words: Nuclear Reprogramming, Induced pluripotent stem cells, Genetic diseases

Introduction

So far, many approaches have been tried to figure out the pathogenesis of genetic diseases, and a large amount of knowledge in this field has been accumulated by tremendous studies via RNA interference (RNAi) techniques and genetic manipulated-mouse models, called knock out (K/O) models. These two systems have mimicked the disease phenotypes *in vitro* and *in vivo* to some extent. However, there are several limitations of these systems in the realization of human disease phenotypes; the cell-based system is hard to reproduce the various cell and/or tissue types *in vitro* and K/O mouse models are occasionally unmatched with human disease phenotypes because of species-specific differences in the anatomic and physiologic characteristics between mouse and human being.[1)] By species-specific differences, the effectiveness of the drugs could be also different in the clinical studies between mouse and human. In fact, many drugs that have been proved to be effective in mouse models do not work well in the patients.[2)] Thus, development of new systems for realizing disease-specific phenotypes is needed to explore fundamental mechanisms of various human diseases. In this context, human embryonic stem cells (hESCs) first established by Thomson et al.[3)] and induced pluripotent stem cells (iPSCs) first developed by Takahashi and Yamanaka[4)] have been highlighted in the research of human diseases at the molecular and cellular levels as well as in the cell replacement therapy. Also, human ESCs and iPSCs can be employed to screen new drugs and to test drug toxicity *in vitro*. In this review, we are focusing on the concept of cellular reprogramming and *in vitro* disease modeling using iPSCs, especially for genetic diseases. Also, we briefly describe current breakthroughs and prospects of the iPSC research.

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Derivation of induced pluripotent stem cells (iPSCs)

Since human ESCs have been generated from human early developing embryos by Thomson and colleagues,[3] new paradigm of disease modeling has been emerged. Genetic defects could be identified from early embryos by pre-implantation genetic diagnosis (PGD) and disease-specific human ESC lines could be generated by homologous recombination capable of manipulating target genes in normal hESCs.[5, 6] However, generation of human ESCs entails strict restrictions, including ethical issues, immune rejection, a paucity of diseases enabling the PGD, and low efficiency of homologous recombination in human ESCs.[7-10] To overcome these barriers, scientists have turned their interests into the cellular reprogramming. So far, several approaches have been tried to induce the cellular reprogramming; somatic cell nuclear transfer (SCNT), culture of somatic cells with nuclear extract of oocytes or ES cells, fusion of a somatic cell with an ES cell, and induction of the pluripotency from differentiated cells by ectopic expression of defined factors (Fig. 1).[11]

SCNT is considered to be a potent cellular reprogramming technique since the Dolly, a first cloned sheep, has been generated from a cloned embryo with a mammary gland cell.[12] To make cloned embryos, somatic cells are individually introduced into enucleated oocytes and then develop to the blastocyst stage. Then, ES-like cells are derived from the cloned embryos. Cloned ESCs can solve the problem of immune rejection in the cell therapy because the genome of cloned ESCs is genetically matched with that of the patient. However, there are a few of limitations in the production of human cloned ESCs. To make human cloned embryos, a lot of human oocytes are required because of low efficiency of cloning, thereby raising serious ethical and social issues. Also, it is not easy to establish human ESC lines from cloned embryos. Stable human ESC lines generated by modified SCNT could normally express pluripotent marker genes and could differentiate into three germ layers, representing that they were pluiripotent *in vitro* and *in vivo*.[13] Nonetheless, those cells showed immature form of DNA methylation and histone modifications, indicating that SCNT in human cells may result in incomplete epigenetic reprogramming. Another approach can be employed to produce pluripotent cells; somatic cells are cultured in the medium containing nuclear extracts of oocytes or ES cells.[11] Hybrid ES-like cells can be generated by fusion of a ESC with a somatic cell using polyethylene glycol (PEG).[14] This method is easy to generate pluripotent cells without raising ethical issues, but there are some limitations such as low efficiency of cell fusion and tetraploidy of fused cells. Most powerful technique is to derive pluripotent stem cells from differentiated cells by ectopic expression of defined factors. This was first developed in 2006 by Dr. Shinya Yamanaka, a Nobel laureate of this year. Briefly, iPSCs could be first generated from mouse fibroblasts by retroviral infection designed for ectopic expression of defined factors such as OCT4, SOX2, KLF4 and cMYC.[4] One year later, human iPSCs could be derived from human dermal fibroblasts by the same method. Thereafter, iPSCs could be generated from various somatic cell types, including blood cells, melanocytes, keratinocytes and stomach cells, representing the universality of cellular reprogramming with this technique.[15-18] However, in case of viral infection which is the most widely used method, random integration of foreign genes into the host genome may give rise to insertional mutagenesis. In addition, incomplete silencing or re-activation of transgenes, especially oncogenes such as c-MYC, may be potentially harmful in case of translational application due to the risk of tumorigenesis.[19] To circumvent these potential risks, many approaches have been developed to generate the safe iPSCs using non-virus systems, including plasmids, proteins, synthetic mRNA and micro RNAs (Fig. 2).[20-26] Furthermore, it has been reported that synthetic small molecules and epigenetic modification agents can partially replace defined factors and improve the efficiency of cellular reprogramming.[27-29]

Recently, the concept of cellular reprogramming is being expanded to a novel technology, called "Direct Conversion". By this approach, specialized cell types could be directly derived from somatic cells without induction of cellular reprogramming to the pluripotent state. As shown in Fig. 3, diverse cell types such as neuronal stem cells, dopaminergic neurons, neuronal cells, cardiomyocytes and blood progenitors could be converted from

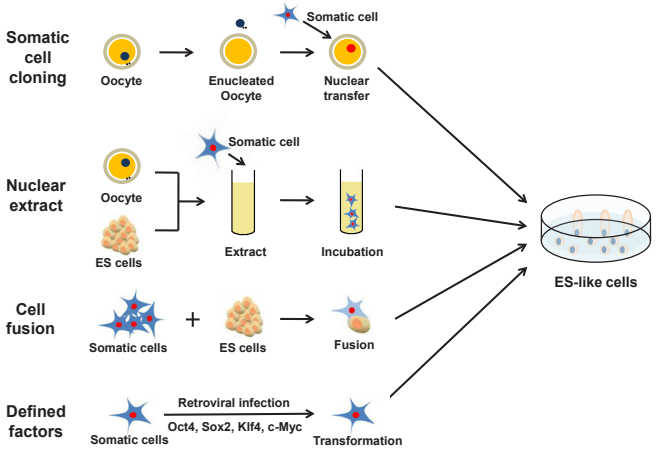

Fig. 1. Several trials for cellular reprogramming.

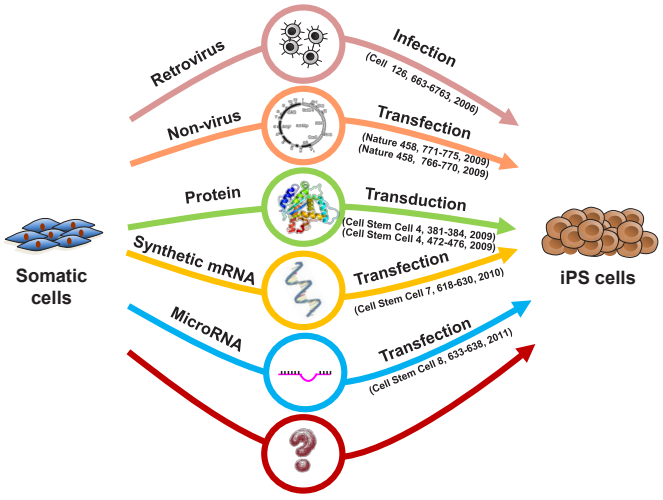

Fig. 2. Various methods for iPSCs generation.

somatic cells by using respective transcription factors which are specifically expressed in a specialized cell type.[30-38] Direct conversion or transdifferentiation will open the new era of cellular reprogramming.

Trends and products of the study in disease-specific iPSC generation

In the past era, it was difficult to recapitulate and demonstrate various disease phenotypes *in vitro* system. Since iPSCs generation as cellular reprogramming was reported successfully by Yamanaka and colleagues, the paradigm *in vitro* study for the genetic diseases was changed revolutionarily and scientists have

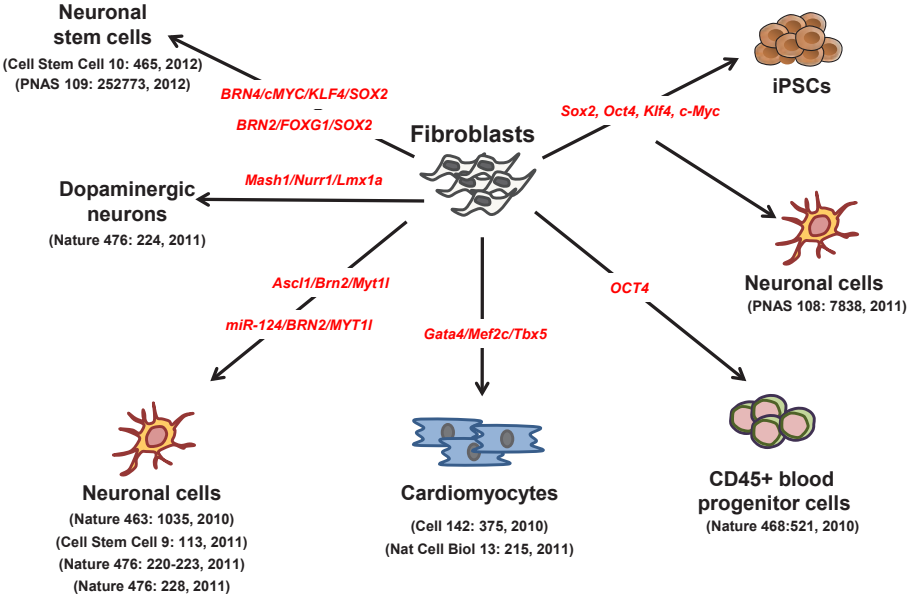

Fig. 3. Direct conversion of somatic cells into specific lineage cells.

Table 1. Examples of Genetic Disease Modeling with Patient-specific iPSCs

Disease	Mutation gene	Relevant cell types	Disease phenocopy	Drug test	References
ALS	*SOD1*	Neuron	X	X	Robinton et al.[39]
FXS	*FMR1*	iPSCs	X	X	Park et al.[40]
LS	*PTPN11*	Cardiomyocyte	O	O	Dimos et al.[41]
FA	*FANCA; FANCD*	Blood progenitor	Gene correction	X	Carvajal-Vergara et al.[43]
SMA	*SMN1; SMN2*	Neuron	O	O	Hanna et al.[44]
LQT	*KCNH2*	Cardiomyocyte	O	O	Itzhaki et al.[51]
TS	*CACNA1C*	Cardiomyocyte	O	O	Raya et al.[45]
SCH	Polygenic	Neuron	O	O	Brennand et al.[52]
ALD	*ABCD1*	Oligodendrocyte	O	O	Jang et al.[53]

ALS, amyotrophic lateral sclerosis; FXS, fragile X syndrome; LS, LEOPARD syndrome; FA, Fanconi anemia; SMA, spinal muscular atrophy; LQTS, long QT syndrome; TS, Timothy syndrome; SCH, schizophrenia; ALD, adrenoleukodystrophy

focused their efforts to the patient-specific iPSCs generation and its disease modeling (Table 1).[39] A variety of human iPSC lines were first derived from 10 patients of degenerative and genetic diseases.[40] Disease-specific iPSCs were generated from dermal fibroblast of ALS (amyotrophic lateral sclerosis) patients and then its specific disease phenotypes could be recapitulated in the differentiated motor neurons and oligodendrocytes.[41] What abnormal expression patterns and epigenetic modification of FMR gene could occur during early developmental period was confirmed by studying iPSCs derived from fragile X syndrome patients.[42] Using iPSCs lines derived from LEOPARD syndrome patients with PTPN11 gene mutation, it was newly suggested that disease phenotype of HCMP (hypertrophic cardiomyopathy) was caused by dysregulation of MAPK pathway and the accumulation of NFTA4 transcription factors in the nucleus of disease-cardiomyocytes.[43]

In addition to the disease modeling via patient-specific iPSCs, many scientists reported the fascinating results in the genetic correction model and cell therapy in disease-iPSCs. Hanna et al. first demonstrated the possibility of cell therapy with iPSCs in mouse model.[44] iPSCs derived from mouse with sickle cell anemia were corrected by using recombination techniques and differentiated into hematopoietic progenitor cells. When disease-corrected hematopoietic progenitors were transplanted into the mouse with sickle cell anemia, the disease phenotypes of sickle cell anemia were rescued by the gene-corrected cells. Correction of human Fanconi anemia-derived iPSCs represented functionally normal phenotypes in blood progenitor cells.[45]

Patient-specific iPSCs could be used to screen new drugs in the pharmaceutical industry. The phenotypes of type I spinal muscular atrophy (SMA) were reproduced in the disease-specific neurons differentiated from SMA patients-specific iPSCs.[46] Then, abnormal phenotypes could be rescued by over-expression of SMN protein in diseased neurons because it was found that reduction of the SMN protein levels resulted in the decrease of motor neuron production and neurite outgrowth. Cardiomyocytes derived from Timothy syndrome-specific iPSCs recapitulated its disease phenotypes including irregular beating, Ca^{2+} over-influx and persistent activated action potential (AP).[47] When CDK inhibitor (roscovitine) to recover abnormal L-type calcium channel (Cav1.2 channel) was treated, abnormal AP and arrhythmia were rescued in the disease-specific cardiomyocytes. These studies demonstrate that human disease-specific iPSCs can complement the paucity of mouse models which are different from human physiologic and pathologic characteristics.

The prospect and application of the disease-specific iPSCs

Among several cellular reprogramming techniques, iPSC generation is free of ethical issues and technically easy. In the early era of the iPSC study, scientists have tried to generate disease-specific iPSCs from patient's somatic cells. Thereafter, the disease modeling that recapitulated the diseased phenotypes in iPSC-derived specialized cell types has been highlighted because the pathogenesis of many genetic diseases could be figured out at the molecular and cellular levels. In addition, iPSCs have a distinct advantage to study the unknown pathogenesis during early human development in many genetic disorders. Now, many researchers have conducted drug screening and *in vitro* pre-clinical study in the disease-specific iPSCs.[48] Also, the cell therapy with disease-corrected iPSCs may be considered as 'patient-specific treatment'.[49] Thus, iPSCs have many advantages to study the diseased phenotypes or the pathogenesis in human during early development. Although disease-specific iPSCs have become a new system to study various genetic diseases, there are still many barriers to be overcome. To investigate various diseases, more efficient techniques of iPSCs generation and robust protocol for differentiation into a specialized cell type should be improved (48). In addition, difficulties to recruit various patients of rare genetic diseases and to perform disease modeling for late onset and multi-factorial diseases are to be solved.[50] Collectively, there is no doubt that disease-specific iPSCs are very useful as attractive materials to study the pathogenesis of genetic diseases at the molecular and cellular levels and to screen new drugs.

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