Induced pluripotent stem (iPS) cell technology involves the generation of pluripotent stem cells from adult somatic cells by the exogenous expression of specific reprogramming factors1,2 (see Box). This technology opened the door for us to access a robust platform demonstrating in vitro cellular reprogramming from certain somatic cells to pluripotent status. Recently, Cahan and Daley8 conducted a bibliometric analysis of the pluripotent stem cells (including iPS cells, embryonic stem [ES] cells, and embryonic carcinoma cells) generated since 2010. They found that the number of publications concerning ES cells has decreased, whereas the number of publications concerning the applications of pluripotent stem cells has increased. They pointed out that it is the increase in the studies of iPS cells that brought about this change in the research trend, because it gave scientists access to pluripotent stem cells with robust and reproducible technology. As Cahan and Daley8 have shown, iPS cells quickly became recognized as an innovative research tool for the study of biology and medicine. In this review, we focus on the cloning event occurring through the reprogramming process and its ability to let us analyze the development of complex disease-harboring somatic mosaicism.

Abstract: Induced pluripotent stem cell technology makes in vitro reprogramming of somatic cells from individuals with various genetic backgrounds possible. By applying this technology, it is possible to produce pluripotent stem cells from biopsy samples of arbitrarily selected individuals with various genetic backgrounds and to subsequently maintain, expand, and stock these cells. From these induced pluripotent stem cells, target cells and tissues can be generated after certain differentiation processes. These target cells/tissues are expected to be useful in regenerative medicine, disease modeling, drug screening, toxicology testing, and proof-of-concept studies in drug development. Therefore, the number of publications concerning induced pluripotent stem cells has recently been increasing rapidly, demonstrating that this technology has begun to infiltrate many aspects of stem cell biology and medical applications. In this review, we discuss the perspectives of induced pluripotent stem cell technology for modeling human diseases. In particular, we focus on the cloning event occurring through the reprogramming process and its ability to let us analyze the development of complex disease-harboring somatic mosaicism. (Circ Res. 2014;114:505-510.)

Key Words: induced pluripotent stem cells • mosaicism
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differentiation protocols yield relatively immature cells.10–12
Cardiomyocytes differentiated from iPS cells using the current
renewable way. However, there are still some limitations that
apply to maintain specific somatic cells on a large scale and in a
proliferative state. These limitations include the difficulty of
preparing large numbers of the desired types of cells from
pluripotent cell lines, a sufficient number of the desired types
of cells can be prepared by using appropriate differentiation
protocols at almost the same time using the same approaches.2,6,7 This
shows that the iPS cell technology was already highly reproduc-
able and accessible to several laboratories and that these cells
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Discovery of Induced Pluripotent Stem
Cells

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cells by the ectopic expression of key transcription factors
(Oct3/4, Sox2, c-Myc, Klf4).1 The iPS cells were indistinguish-
able from embryonic stem cells in terms of their morphol-
gene expression, self-renewal capacity, and pluripotency.
Therefore, under this process, the somatic memory of the sub-
ject cells was reprogrammed to the stage of blastocyst inner
cells. Further experiments confirmed the contribution of iPS
cells to mammalian (mouse) ontogenesis. The mouse iPS cells
have been confirmed to have a chimeric contribution following
blastocyst injection and have also been proven to have compet-
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Subsequently, human iPS cells were established by 3 laborato-
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iPS Cells for In Vitro Modeling of Native
Physiological Conditions

Immortalized cells and primary cell lines have been the candi-
date cells used for creating human physiological models thus
far. However, both of these types of cells are associated with
serious defects when used for such purposes. Although immor-
talized cell lines can be maintained as homogenous cell
populations at low cost, they lack important aspects of the na-
tive functions of cells. Likewise, although primary cell lines
provide fully differentiated cells that have a close approxima-
tion of the native function, the collection of all cell types is
not possible because of the level of invasion required to collect
them, and the limited proliferative activity of primary cells
can make reproducing experiments difficult.6

On the contrary, human-derived iPS and ES cell lines can
overcome these disadvantages. After the expansion of these
pluripotent cell lines, a sufficient number of the desired types
of cells can be prepared by using appropriate differentiation
protocols, that is, these technologies can provide an opportu-
nity to maintain specific somatic cells on a large scale and in a
renewable way. However, there are still some limitations that
need to be overcome. For example, it has been pointed out that
cardiomyocytes differentiated from iPS cells using the current
differentiation protocols yield relatively immature cells.10–12
Therefore, further improvement of the differentiation and
maturation protocols will be needed to more accurately reca-
pitulate the phenotype in iPS cell–derived differentiated cells.

Human ES cells carrying mutation for some genetic disor-
ders have been reported (e.g., Huntington disease, cystic fibro-
sis, myotonic dystrophy type 1, and Fragile X syndrome).13,14
However, there are certain ethical concerns associated with
developing ES cells obtained from in vitro fertilization clinics
for the study of genetic diseases.15 In contrast, such ethical
issues are overcome with the use of disease-specific iPS cell
lines, because they can be generated from somatic cells such as
fibroblasts or peripheral blood cells, which can be collected
by minimally invasive procedures and do not require the de-
struction of a human embryo.

iPS Cells From Arbitrarily Selected Individuals

Another advantage of using iPS cells in medical research is
that this technology makes it possible to obtain human plu-
ripotent stem cells from arbitrarily selected individuals with
various genetic backgrounds, including patients with various
diseases. In 2008, the first patient-specific iPS cells were gen-
erated.16,17 Since then, abundant studies of disease models that
represent the human condition with high fidelity have been
reported,18,19 particularly those aiming to investigate diseases
that are caused by a single gene mutation. In most of the stud-
ies, iPS cell lines from unaffected or healthy donors were used
as controls. However, recent genome-wide association stud-
ies using a set of controls derived from different individuals
showed significant experimental noise attributable to genomic
variations. Moreover, these controls cannot really be defined
as healthy, because each person carries 50 to 100 disease-
associated genetic variations.20 One way to address these is-
ues is to obtain isogenic controls by correcting mutated genes
in patient-specific iPS cell lines.21–27 The recent progresses
in genetic engineering technologies, such as Transcription
Activator-Like Effector Nuclease (TALEN) and Clustered
Regularly Interspaced Short Palindromic Repeats (CRISPRs),
will facilitate this strategy.27,28

Somatic mutations can give rise to a broad range of diseases,
including cancer, and noncancerous mosaic diseases.29 Recently,
heterogeneous patient-specific iPS cell lines have been generated
from the somatic cells of patients with various mosaic diseases,
such as Down syndrome (DS),30–35 Fragile X syndrome,36 Rett
syndrome,37–42 Fanconi anemia,43 and chronic infantile neurolog-
ic cutaneous and articular (CINCA) syndrome.44 Even though
there are some limitations associated with the kinds of diseases
that can be modeled, using iPS cells derived from patients with
somatic mosaicism has an advantage in that it allows analyses
with isogenic controls. In these cases, both mutant and wild-type
iPS cell clones can be generated from the same patients. Each
pair of clones theoretically has the same genetic background,
except for the altered chromosome or mutated gene(s) and should
serve as an ideal pair of mutant and isogenic control clones,
thereby allowing for highly reliable studies of the impact of the
mutation. It should be mentioned that we could not completely
exclude the introduction of new mutations during the induction
of iPS cells and subsequent cultivation. The recently improved
next-generation DNA sequencing and bioinformatics techniques
should be useful in detecting such acquired mutations and in-
creasing the quality of experiments.

Nonstandard Abbreviations and Acronyms

CINCA  chronic infantile neurologic cutaneous and articular
DS  Down syndrome
ES  embryonic stem
IPS  induced pluripotent stem

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exception that the iPS cells are not derived from embryos.
The following examples of patient-specific iPS cells generated from mosaic cases of DS and CINCA syndrome illustrate the advantage of using iPS cell technology for disease modeling.

**Somatic Mosaicism in DS**

DS is the most frequent form of mental retardation and is caused by autosomal trisomy of all or a critical portion of chromosome 21. Patients with DS are reported to present with multiple disorders (eg, congenital heart defects, particularly atrioventricular septal defect, leukemia, and early-onset Alzheimer disease [OMIM 190685]). A cytogenetic study showed that, in the United States, most infants with DS (≈95%) had full trisomy 21; in ≈3% of patients, one copy was translocated to another acrocentric chromosome, and in ≈2% of cases, live-born trisomy 21 individuals were recognized to have mosaicism for a trisomic cell line. Mosaicism results from the abnormal division of some cells after fertilization, with some of the cells having 47 chromosomes and the others being normal. In each of these conditions, the outcome of this disorder is determined by the particular chromosome being duplicated, as well as by the proportion of cells in the body carrying the abnormality.

Papavassiliou et al observed a correlation between the frequency of trisomic cells and the patient phenotype. They detected a significant inverse correlation between the frequency of trisomic cells and IQ scores in individuals with mosaic trisomy 21. Mosaic trisomies have also been reported on other chromosomes, such as chromosomes 1, 8, 9, 13, 16, 17, 18, and 22 (as reviewed by Poduri et al). Many attempts have been made to model DS to investigate the detailed pathophysiology of the condition. Generally, biopsy samples of brains are not available from patients, and thus, mouse models are currently the primary tools used to study the pathogenesis of DS. However, brain development differs in mice and humans. Although some human ES cells with trisomy 21 have been generated, the establishment of human ES cells from patients with DS remains ethically challenging.

**Capturing Trisomic and Disomic Cells From Mosaic Patients Using the iPS Cell Technology: DS as an Example**

DS/trisomy 21 patient-specific iPS cell lines have been generated from various cases, including mosaic patients. Recently, 2 groups reported the generation of iPS cell lines from fibroblasts that were derived from patients with DS with mosaicism. They confirmed that the trisomy 21 karyotype was present in the DS iPS cells; however, they also captured iPS cell lines from the patient’s fibroblasts, which were found to possess a disomic karyotype. This seemed to be the result of the reprogramming of euploid cells from the mosaic donor’s fibroblast population. DNA profiling of the corresponding cell lines revealed that these disomic and trisomic cell lines actually came from the same donor. The patient’s disomic cell line provides an ideal experimental control, because it can be used to cancel the noise in the genetic background that is often observed using so-called “healthy” individuals as controls. This system presents a powerful method for analyzing multiple genotype-phenotype associations in complex diseases that harbor multiple candidate mutations, such as chromosomal aberrations (Figure).

**Somatic Mosaicism in CINCA Syndrome**

Beside DS, patient-specific iPS cell lines have also been generated from the somatic cells of patients with autosomal mosaic point mutations. For example, CINCA syndrome is a dominantly inherited autoinflammatory disease characterized by cutaneous symptoms, central nervous system involvement, and arthropathy (OMIM 607115). Saito et al described the first case of a patient with somatic mosaicism in their NLRP3 (also known as CIAS1) gene mutation. It has been observed that 30% to 40% of all patients have mutations in NLRP3 in only a small number (≈10%) of somatic cells, despite the fact that nearly half of all patients with CINCA syndrome carry heterozygous gain-of-function mutations of the gene. Recently, Tanaka et al generated both NLRP3 mutant and nonmutant iPS cell lines from patients with CINCA syndrome with somatic mosaicism and described their differentiation into macrophages. In this case, they succeeded in recapitulating the disease-relevant phenotype using only mutant macrophages derived from iPS cells, demonstrating that NLRP3-mutant macrophages are responsible for the pathogenesis of mosaic CINCA syndrome. Moreover, they succeeded in developing a drug screening system with the macrophages derived from iPS cells and illustrated the usefulness of iPS cell technology as a platform for drug discovery.

**A New Approach to Studying Somatic Mutations Using the iPS Cell Technology**

Recently, somatic mosaicism has been reported in a variety of tissues from healthy individuals, suggesting that it has physiological functions. In such cases, iPS cell technology can provide a universally applicable strategy to capture these somatic mosaicsisms in human populations, allowing their impact to be evaluated.

Evidence from recent studies using single-nucleotide polymorphism microarrays or the next-generation sequencing technology demonstrates that a certain proportion of mutations that are detected in iPS cells come from the heterogeneous donor cell population, attributable to somatic mosaicism. The cells that carry pre-existing genetic alterations, such as single-nucleotide variants and copy number variations, are captured by the cloning event through the reprogramming process.
These single-nucleotide variants and copy number variations in a minor population of somatic cells have to date been overlooked because of the difficulty in detecting them. However, applying iPS cell technology allows for improved detection of such low-level mosaicism.

**Somatic Mosaicism in Cardiovascular Diseases**

Systematic analyses of Leipzig heart collections have shown somatic mutations in several transcription factor genes (NKX2-5, TBX5, GATA4, and HEY2) associated with complex congenital heart diseases, including atrial septal, ventricular, and atrioventricular septal defects.\(^{56–64}\) In these cases, each mutation was detected in the affected heart tissues, but not in the normal heart tissues, of the same patients. In this situation, mutant cardiomyocytes can be provided by gene editing of wild-type iPS cell lines.

Another set of examples of mosaicism was found in somatic mutations associated with arrhythmia.\(^{62–64}\) Mosaicism in connexin 40 and connexin 43 is associated with atrial fibrillation.\(^{63,64}\) Interestingly, subcloning analysis estimated allelic frequencies of mutant alleles at 20% to 34% within the patient’s cardiac tissue specimens; however, these mutations were not detected in lymphocytes. Each mutant connexin-expressing cell was observed loss of contribution to gap junction formations, as well as electrophysiological functions.

To further investigate the pathophysiology of those atrial fibrillations with somatic mosaicism of connexins, modeling of in vitro cardiac syncytia in which each cardiomyocyte is individually expressing either wild-type or mutant connexins provides a powerful tool. Recently, Kadota et al\(^{65}\) created cardiomyocyte sheets from human iPS/ES cells. They demonstrated these sheets are capable of generating re-entrant arrhythmia models when stimulated with high-frequency electric pulses and subsequently showed their usefulness for screening and testing drugs with antiarrhythmic potential. Although further technological improvements are needed, this approach can provide a more precise in vitro micro-re-entrant model for the mosaic cardiac syncytium. In this case, we may choose to obtain mutated cardiac cells by gene-editing technology based on unaffected/well-characterized iPS cells because to date mutant connexin 40–expressing and connexin 43–expressing cells have only been detected in patient cardiac tissues, which are difficult to obtain by biopsy.

**Concluding Remarks**

Somatic variants are known to be potentially responsible for various diseases. However, the extent of somatic variation may have been markedly underestimated.\(^{66}\)

As we discussed above, iPS cell technology allows us to approach human biology with higher resolution at the cellular level through the cell cloning process, as part of the reprogramming process. This approach holds great promise for studies on the native human physiological processes, as well as the development and pathology of human diseases. For example, in patients with trisomy 21, acquired mosaicism was observed in adult patients.\(^{67}\)

Although there are still several technical hurdles that have to be overcome, we may be able to analyze such a disease/environmental/aging association successfully at the molecular level by applying iPS cell technology. Moreover, iPS cell technology holds the potential to lead to new insights into the human physiology associated with somatic mosaicism.

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**References**


Perspectives for Induced Pluripotent Stem Cell Technology: New Insights Into Human Physiology Involved in Somatic Mosaicism

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