Induced pluripotent stem cells (iPSCs) are potential cell sources for regenerative medicine and other clinical applications, such as cell therapies, drug screening, toxicology testing, and the investigation of disease mechanisms. Discovery of disease-associated iPSCs has led to the development of a new field of disease modeling, as they can provide somatic cells which cannot be directly obtained from each patient. In this review, we focus on the applications of disease-associated iPSCs for understanding human hematological and immunological disorders, while discussing the current state of hematopoietic differentiation and the findings of previous reports of disease-associated iPSCs in this field.

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**Introduction**

Hematological and immunological disorders are abnormalities of the blood systems. Although elucidation of their cellular pathophysiology has been largely based on in vitro studies using patient-derived primary hematopoietic cells or animal models, these approaches have potential limitations. For example, patient-derived cells cannot be obtained in unlimited quantities, and their in vitro functions can be affected by in vivo conditions, such as the cytokine milieu or therapeutic agents. Furthermore, in the case of multi-system disorders, such as those associated with chromosomal abnormalities, defects in DNA repair, or metabolic disorders, obtaining patient-derived samples other than blood is difficult, hampering the analysis of affected cells or tissues. On the other hand, although murine models have provided important insights into various disorders, differences in the hematological and immunological development between mice and humans sometimes causes discrepancies in the resulting phenotypes.

Because of their pluripotency and capacity for self-renewal, human pluripotent stem cells (PSCs), such as embryonic stem cells (ESCs) and induced pluripotent stem
cells (iPSCs) are potential sources of cells for regenerative medicine and other clinical applications, such as cell therapies, drug screening, toxicology testing, and investigation of disease mechanisms. iPSCs, first established by Takahashi and Yamanaka, are reprogrammed somatic cells with ESC-like characteristics that are generated by introducing certain transcriptional factors such as OCT3/4, SOX2, KLF4 and cMYC\(^6\). These transcriptional factors can be delivered into the source cells by viral vectors, episomal vectors\(^6\), piggybac transposon\(^6\) or modified synthetic RNA\(^6\). Discovery of disease-associated iPSCs has led to the development of a new field of disease modeling, as they can provide somatic cells which cannot be directly obtained from each patient.

**Directed differentiation into hematopoietic cells from human PSCs**

Although patient- or disease-specific iPSCs are an important resource for unraveling human hematological disorders, a robust and simple hematopoietic differentiation system that can reliably mimic in vivo hematopoiesis is necessary for this purpose. The leading methods of hematopoietic cell induction from PSCs employ two different systems: namely, monolayer animal-derived stromal cell co-culture and three-dimensional embryoid body (EB) formation. Both methods can produce hematopoietic cells from mesodermal progenitors, and combinations of cytokines can control, at least to some extent, the specific lineage commitment\(^11\). We recently established a robust and simple monolayer hematopoietic cell differentiation system from human PSCs\(^20\). Our system is free from xeno-feeder cells or serum, and can trace the in vitro differentiation of human PSCs into multiple lineages of definitive blood cells, such as functional erythrocytes and neutrophils.

Because human PSCs are feasible cell sources for various clinical applications, the scientific and medical communities have shown continuing interest in hematopoietic stem cell (HSC) induction from PSCs. Previous trials have indicated that murine ESC-derived hematopoietic cells overexpressing HoxB4 resulted in long-term myelo-lymphoid reconstitution in the bone marrow of lethally irradiated recipient mice\(^31\). However, the transduction of HOXB4 was not sufficient to develop fully functional human HSCs\(^22\), and it remains a challenge to develop bona fide human HSCs with bone marrow reconstitution activity at the single-cell level.

Despite the recent advances, the directed differentiation of human PSCs into definitive hematopoietic cells in vitro is also still challenging. Most cultures develop into mostly nucleated erythrocytes with a primitive or definitive fatal type hemoglobin expression pattern (\(\alpha\)- and \(\gamma\)-globins), and the robust and effective differentiation of enucleated adult type \(\alpha\)- and \(\beta\)-globin-expressing red blood cells from human PSCs remains elusive. The differentiation of lymphoid cells is also relatively difficult. While T-lymphocytes can be derived from human PSCs on OP9-DL1 feeder layers\(^19\), the terminal differentiation into B-lymphocytes remains to be accomplished, because it has so far not been possible to make cells go through the pre-B state\(^20\). Several groups have reported the derivation of functional natural killer cells from PSCs\(^24,28\).

**Disease-associated iPSCs from patients with hematological or immunological disorders\(^26-45\)**

A number of disease-associated iPSCs generated from patients with hematological or immunological disorders have been reported (Table 1). However, several papers just reported the establishment of iPSC clones, while discussing the potential usefulness of disease-associated iPSCs as a resource for disease analysis. The first report of disease-associated iPSCs derived from patients with a hematological disorder was iPSCs from Fanconi anemia (FA)\(^27\). Raya et al. established FA patient-derived iPSC clones from Fanconi anemia patients after correcting genetic defects in the parental fibroblasts. Uncorrected fibroblast could not be reprogrammed into iPSCs, indicating that the Fanconi anemia pathway is requisite for reprogramming. The corrected iPSC clones could differentiate into hematopoietic cells normally. Recently, another group found that iPSCs from Fanconi anemia patients could be generated without complementation, although the efficiency was extremely low\(^25\).

For \(\beta\)-thalassemia, one of the most common hereditary anemias\(^46\), disease-associated iPSC studies have been mainly conducted towards proving that the iPSC technology could be used to generate gene-corrected cells with potential value for cell therapy. Wang et al., genetically corrected iPSCs from a \(\beta\)-thalassemia patient by homologous recombination, and differentiated them into hematopoietic progenitors\(^29,31\). When the cells were transferred to sub-lethally irradiated NOD/SCID mice, the hemoglobin
level recovered efficiently. Papapetrou et al. showed that transduction of a lentivirally encoded β-globin transgene into genomic safe harbors enabled high expression of the transgene in β-thalassemia iPSC-derived erythroid progenitors. Similar to β-thalassemia, two groups reported genetic correction using cells from patients with sickle cell anemia, 33,35.

Regarding immunological disorders, two groups recently established iPSCs from patients with chronic granulomatous disease (CGD), 16, 37, a primary immunodeficiency characterized by impaired phagocytic killing of microorganisms by neutrophils and macrophages. 47. Both groups demonstrated that the differentiated neutrophils from disease-associated iPSCs lack the production of reactive oxygen species (ROS) in response to proper stimulus. As a model for gene therapy, Zou et al. restored the neutrophil ROS production in X-linked CGD iPSCs by zinc finger nuclease-mediated gene targeting of a single-copy gp91phox therapeutic minigene into one allele of the “safe harbor” AAVS1 locus. 37.

Disease-associated iPSCs from patients with chronic myeloid leukemia (CML) have also been reported. The sources of iPSCs were a cell line, KBM7, primary bone marrow cells, and CD34+ cells. All iPSC clones bore the translocation of 9;22 breakpoints of the BCR/ABL fusion gene. Interestingly, although the parental cell lines were sensitive to the tyrosine kinase inhibitor imatinib, thus showing their dependency on BCR/ABL oncogene signaling, a loss of oncogene addiction was observed in the reprogrammed iPSC clones. The hematopoietic differen-

Table 1 Reported disease-associated iPSC cell lines representing hematological and immunological disorders

<table>
<thead>
<tr>
<th>Category</th>
<th>Name of disorder</th>
<th>Molecular defect</th>
<th>Author</th>
<th>Year</th>
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*, ** Reported by the same group.
Limitations and unsolved issues for studies of disease-specific iPSCs

As discussed above, most of the previously reported disease-associated iPSC studies have been just “disease-modeling” or proof-of-principle studies. To gain more insight into disease pathophysiology by using iPSC technology, several issues still need to be overcome. One of the most critical issues is to develop a method to obtain mature, fully functional hematopoietic cells, including HSCs. Another concern is that, even if iPSCs are obtained from an individual, the differentiation efficiency and/or functions of hematopoietic cells will show inter-clonal variation, which hampers the accurate estimation of the disease-associated phenotypes of patient-derived iPSCs. These variations may derive from inter-clonal genetic variations\(^{51}\), epigenetic modifications\(^{52}\), the source of iPSCs, residual transgenes of each iPSC clone, or, in female cases, the alteration of the status of X chromosome inactivation\(^{53, 54}\). Additionally, fibroblasts obtained from patients with certain diseases, such as Fanconi anemia\(^{27}\) and dyskeratosis congenita\(^{53}\), show extremely low reprogramming efficiency. For these types of diseases, a specific reprogramming strategy, such as transient genetic complementation, may therefore be required.

Conclusion

Although disease-associated iPSCs are useful tools, their proper differentiation into functional hematopoietic cells is essential for elucidating the cellular pathophysiology of hematopoietic and immunological diseases. The establishment of suitable disease models that can represent the in vivo phenotype is also important. Rapid technological advances in iPSCs and their differentiation will open up a new horizon for studies that can aid in understanding human diseases.

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Conflict of interest

The authors declare no conflict of interest.

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