Inflammation and Regeneration Vol.32 No.4 SEPTEMBER 2012

Special Issue: Hematopoietic and Mesenchymal Stem Cells

Mini Review

Disease-associated iPS cell lines representing hematological and immunological disorders

Megumu K. Saito* and Akira Niwa

Department of Clinical Application, Center for iPS cell research and application, Kyoto University, Kyoto, Japan

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.

Rec.7/2/2012, Acc.8/13/2012, pp171-177

*Correspondence should be addressed to:
Megumu K. Saito, Department of Clinical Application, Center for iPS cell research and application, Kyoto University, 53 Shogoin-Kawahara, Sakyo, Kyoto 606-8507, Japan. Phone: +81-75-366-7085, Fax: +81-75-366-7091, E-mail: msaito@cira.kyoto-u.ac.jp

Key words induced pluripotent stem cells, hematopoietic differentiation, hematological disorders, immunological disorders

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 multisystem disorders, such as those associated with chromosomal abnormalities[1], defects in DNA repair[2], or metabolic disorders[3], 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[4, 5]. 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, 7]. These transcriptional factors can be delivered into the source cells by viral vectors, episomal vectors[8], piggybac transposon[9] or modified synthetic RNA[10]. 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[11-19]. 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[21]. 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 (α- and γ-globins), and the robust and effective derivation of enucleated adult type α- and β-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[23]. Several groups have reported the derivation of functional natural killer cells from PSCs[24, 25].

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[26].

For β-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 β-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

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

Category	Name of disorder	Molecular defect	Author	Year	Reference
Bone marrow failure	Fanconi anemia	*FANCA/FANCC*	Muller	2012	26)
Bone marrow failure	Fanconi anemia	*FANCA/FANCD2*	Raya	2009	27)
Bone marrow failure	Swachman-Bodian-Diamond syndrome	*SBDS*	Park	2008	42)
Bone marrow failure	Dyskeratosis congenita	*DKC*	Batista	2011	43)
Chromosomal abnormality	Down syndrome	Trisomy 21	Park	2008	42)
Hemoglobinopathy	Sickle cell anemia	*HBB*	Sebastiano	2011	33)
Hemoglobinopathy	Sickle cell anemia	*HBB*	Mali*	2008	35)
Hemoglobinopathy	Sickle cell anemia	*HBB*	Zou*	2012	34)
Hereditary anemia	beta-thalassemia	*HBB*	Ye	2009	28)
Hereditary anemia	beta-thalassemia	*HBB*	Wang**	2009	29)
Hereditary anemia	beta-thalassemia	*HBB*	Papapetrou	2011	30)
Hereditary anemia	beta-thalassemia	*HBB*	Wang**	2012	31)
Hereditary anemia	beta-thalassemia	*HBB*	Fan	2012	32)
Hematological malignancy	Chronic myeloid leukemia	*BCR/ABL*	Hu	2011	38)
Hematological malignancy	Chronic myeloid leukemia	*BCR/ABL*	Carette	2011	39)
Hematological malignancy	Chronic myeloid leukemia	*BCR/ABL*	Kumano	2012	40)
Rare blood type	Rare ABO blood type (Bombey)	*FUT1/FUT2*	Seifinejad	2009	45)
Immunodeficiency	Chronic granulomatous disease	*p47phox, qp91phox*	Jiang	2012	36)
Immunodeficiency	Chronic granulomatous disease	*gp91phox*	Zou	2011	37)
Immunodeficiency	Severe combined immunodeficiency	*ADA*	Park	2008	42)
Immunodeficiency	Severe combined immunodeficiency	*RAG1*	Pessach	2010	44)
Immunodeficiency	Omenn syndrome	*RAG1*	Pessach	2010	44)
Immunodeficiency	Herpes simplex type 1 encephalitis	*STAT1*	Pessach	2010	44)
Immunodeficiency	Herpes simplex type 2 encephalitis	*LR3*	Pessach	2010	44)
Immunodeficiency	Cartilage hair hypoplasia	*RMRP*	Pessach	2010	44)
Autoinflammatory syndrome	CINCA syndrome	*NLRP3*	Tanaka	2012	41)

*, ** Reported by the same group.

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[30]. 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)[36, 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[38-40]. The sources of iPSCs were a cell line, KBM7[39], primary bone marrow cells[38], and CD34+ cells[40]. 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[40]. The hematopoietic differen-

tiation of these iPSCs recovered their sensitivity to imatinib.

One of the interesting characteristics of iPSCs is that each iPSC clone originates from a single somatic cell[48]. By taking advantage of this, we have proposed that iPSC technology can be used to dissect and evaluate the genetically different somatic cells from an individual[41]. In CINCA syndrome, an autoinflammatory syndrome caused by mutations of the *NLRP3* gene, 30 to 40% of patients have mutations in *NLRP3* in only a small number of somatic cells[49, 50], and it remains controversial whether the small fraction of *NLRP3*-mutated cells actually causes the strong autoinflammation in these patients, or if all cells carry an unknown mutation of another gene that causes the disease. To resolve this controversy, mutant and non-mutant iPSC lines were established from the CINCA patients with somatic mosaicism. By analyzing the disease-relevant characteristics of IL-1β secretion from the iPSC-derived macrophages, we demonstrated that mutant macrophages are mainly responsible for the disease phenotype in the mosaic patients, confirming the role of *NLRP3*.

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[43], show extremely low reprogramming efficiency. For these types of diseases, a specific reprograming 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.

Acknowledgements

This work was partly supported from a grant from the Ministry of Education, Culture, Sports, Science and Technology (MEXT).

Conflict of interest

The authors declare no conflict of interest.

References

1) Bruwier A, Chantrain CF: Hematological disorders and leukemia in children with Down syndrome. Eur J Pediatr. 2012; 171: 1301-1307.
2) Deans AJ, West SC: DNA interstrand crosslink repair and cancer. Nat Rev Cancer. 2011; 11: 467-480.
3) Stoffels M, Simon A: Hyper-IgD syndrome or mevalonate kinase deficiency. Curr Opin Rheumatol. 2011; 23: 419-423.
4) Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM: Embryonic stem cell lines derived from human blastocysts. Science. 1998; 282: 1145-1147.
5) Keller G: Embryonic stem cell differentiation: emergence of a new era in biology and medicine. Genes Dev. 2005; 19: 1129-1155.
6) Takahashi K, Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006; 126: 663-676.
7) Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S: Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007; 131: 861-872.
8) Okita K, Matsumura Y, Sato Y, Okada A, Morizane A, Okamoto S, Hong H, Nakagawa M, Tanabe K, Tezuka K, Shibata T, Kunisada T, Takahashi M, Takahashi J, Saji H, Yamanaka S: A more efficient method to generate integration-free human iPS cells. Nat Methods.

2011; 8: 409-412.
9) Woltjen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, Hämäläinen R, Cowling R, Wang W, Liu P, Gertsenstein M, Kaji K, Sung HK, Nagy A: piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature. 2009; 458: 766-770.
10) Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, Ebina W, Mandal PK, Smith ZD, Meissner A, Daley GQ, Brack AS, Collins JJ, Cowan C, Schlaeger TM, Rossi DJ: Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell. 2010; 7: 618-630.
11) Umeda K, Heike T, Yoshimoto M, Shiota M, Suemori H, Luo HY, Chui DH, Torii R, Shibuya M, Nakatsuji N, Nakahata T: Development of primitive and definitive hematopoiesis from nonhuman primate embryonic stem cells in vitro. Development. 2004; 131: 1869-1879.
12) Umeda K, Heike T, Yoshimoto M, Shinoda G, Shiota M, Suemori H, Luo HY, Chui DH, Torii R, Shibuya M, Nakatsuji N, Nakahata T: Identification and characterization of hemoangiogenic progenitors during cynomolgus monkey embryonic stem cell differentiation. Stem Cells. 2006; 24: 1348-1358.
13) Ji P, Jayapal SR, Lodish HF: Enucleation of cultured mouse fetal erythroblasts requires Rac GTPases and mDia2. Nat Cell Biol. 2008; 10: 314-321.
14) Vodyanik MA, Bork JA, Thomson JA, Slukvin II: Human embryonic stem cell-derived CD34+ cells: efficient production in the coculture with OP9 stromal cells and analysis of lymphohematopoietic potential. Blood. 2005; 105: 617-626.
15) Kitajima K, Tanaka M, Zheng J, Yen H, Sato A, Sugiyama D, Umehara H, Sakai E, Nakano T: Redirecting differentiation of hematopoietic progenitors by a transcription factor, GATA-2. Blood. 2006; 107: 1857-1863.
16) Takayama N, Nishikii H, Usui J, Tsukui H, Sawaguchi A, Hiroyama T, Eto K, Nakauchi H: Generation of functional platelets from human embryonic stem cells in vitro via ES-sacs, VEGF-promoted structures that concentrate hematopoietic progenitors. Blood. 2008; 111: 5298-5306.
17) Choi KD, Vodyanik MA, Slukvin II: Generation of mature human myelomonocytic cells through expansion and differentiation of pluripotent stem cell-derived lin-CD34+CD43+CD45+ progenitors. J Clin Invest. 2009; 119: 2818-2829.
18) Niwa A, Umeda K, Chang H, Saito M, Okita K, Takahashi K, Nakagawa M, Yamanaka S, Nakahata T, Heike T: Orderly hematopoietic development of induced pluripotent stem cells via Flk-1(+) hemoangiogenic progenitors. J Cell Physiol. 2009; 221: 367-377.
19) Timmermans F, Velghe I, Vanwalleghem L, De Smedt M, Van Coppernolle S, Taghon T, Moore HD, Leclercq G, Langerak AW, Kerre T, Plum J, Vandekerckhove B: Generation of T cells from human embryonic stem cell-derived hematopoietic zones. J Immunol. 2009; 182: 6879-6888.
20) Niwa A, Heike T, Umeda K, Oshima K, Kato I, Sakai H, Suemori H, Nakahata T, Saito MK: A novel serum-free monolayer culture for orderly hematopoietic differentiation of human pluripotent cells via mesodermal progenitors. PLoS One. 2011; 6: e22261.
21) Kyba M, Perlingeiro RC, Daley GQ: HoxB4 confers definitive lymphoid-myeloid engraftment potential on embryonic stem cell and yolk sac hematopoietic progenitors. Cell. 2002; 109: 29-37.
22) Schiedlmeier B, Klump H, Will E, Arman-Kalcek G, Li Z, Wang Z, Rimek A, Friel J, Baum C, Ostertag W: High-level ectopic HOXB4 expression confers a profound in vivo competitive growth advantage on human cord blood CD34+ cells, but impairs lymphomyeloid differentiation. Blood. 2003; 101: 1759-1768.
23) Carpenter L, Malladi R, Yang CT, French A, Pilkington KJ, Forsey RW, Sloane-Stanley J, Silk KM, Davies TJ, Fairchild PJ, Enver T, Watt SM: Human induced pluripotent stem cells are capable of B-cell lymphopoiesis. Blood. 2011; 117: 4008-4011.
24) Vodyanik MA, Bork JA, Thomson JA, Slukvin II: Human embryonic stem cell-derived CD34+ cells: efficient production in the coculture with OP9 stromal cells and analysis of lymphohematopoietic potential. Blood. 2005; 105: 617-626.
25) Ni Z, Knorr DA, Clouser CL, Hexum MK, Southern P, Mansky LM, Park IH, Kaufman DS: Human pluripotent stem cells produce natural killer cells that mediate anti-HIV-1 activity by utilizing diverse cellular mechanisms. J Virol. 2011; 85: 43-50.
26) Müller LU, Milsom MD, Harris CE, Vyas R, Brumme KM, Parmar K, Moreau LA, Schambach A, Park IH,

London WB, Strait K, Schlaeger T, Devine AL, Grassman E, D'Andrea A, Daley GQ, Williams DA: Overcoming Reprogramming Resistance of Fanconi Anemia Cells. Blood. 2012; 119: 5449-5457.

27) Raya A, Rodríguez-Pizàl, Guenechea G, Vassena R, Navarro S, Barrero MJ, Consiglio A, Castellà M, Río P, Sleep E, González F, Tiscornia G, Garreta E, Aasen T, Veiga A, Verma IM, Surrallés J, Bueren J, Izpisúa Belmonte JC: Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. Nature. 2009; 460: 53-59.

28) Ye L, Chang JC, Lin C, Sun X, Yu J, Kan YW: Induced pluripotent stem cells offer new approach to therapy in thalassemia and sickle cell anemia and option in prenatal diagnosis in genetic diseases. Proc Natl Acad Sci USA. 2009; 106: 9826-9830.

29) Wang Y, Jiang Y, Liu S, Sun X, Gao S: Generation of induced pluripotent stem cells from human beta-thalassemia fibroblast cells. Cell Res. 2009; 19: 1120-1123.

30) Papapetrou EP, Lee G, Malani N, Setty M, Riviere I, Tirunagari LM, Kadota K, Roth SL, Giardina P, Viale A, Leslie C, Bushman FD, Studer L, Sadelain M: Genomic safe harbors permit high beta-globin transgene expression in thalassemia induced pluripotent stem cells. Nat Biotechnol. 2011; 29: 73-78.

31) Wang Y, Zheng CG, Jiang Y, Zhang J, Chen J, Yao C, Zhao Q, Liu S, Chen K, Du J, Yang Z, Gao S: Genetic correction of beta-thalassemia patient-specific iPS cells and its use in improving hemoglobin production in irradiated SCID mice. Cell Res. 2012; 22: 637-648.

32) Fan Y, Luo Y, Chen X, Li Q, Sun X: Generation of Human beta-thalassemia Induced Pluripotent Stem Cells from Amniotic Fluid Cells Using a Single Excisable Lentiviral Stem Cell Cassette. J Reprod Dev. 2012; 58: 404-409.

33) Sebastiano V, Maeder ML, Angstman JF, Haddad B, Khayter C, Yeo DT, Goodwin MJ, Hawkins JS, Ramirez CL, Batista LF, Artandi SE, Wernig M, Joung JK: In situ genetic correction of the sickle cell anemia mutation in human induced pluripotent stem cells using engineered zinc finger nucleases. Stem Cells. 2011; 29: 1717-1726.

34) Zou J, Mali P, Huang X, Dowey SN, Cheng L: Site-specific gene correction of a point mutation in human iPS cells derived from an adult patient with sickle cell disease. Blood. 2011; 118: 4599-4608.

35) Mali P, Ye Z, Hommond HH, Yu X, Lin J, Chen G, Zou J, Cheng L: Improved efficiency and pace of generating induced pluripotent stem cells from human adult and fetal fibroblasts. Stem Cells. 2008; 26: 1998-2005.

36) Jiang Y, Cowley SA, Siler U, Melguizo D, Tilgner K, Browne C, Dewilton A, Przyborski S, Saretzki G, James WS, Seger RA, Reichenbach J, Lako M, Armstrong L: Derivation and functional analysis of patient-specific induced pluripotent stem cells as an in vitro model of chronic granulomatous disease. Stem Cells. 2012; 30: 599-611.

37) Zou J, Sweeney CL, Chou BK, Choi U, Pan J, Wang H, Dowey SN, Cheng L, Malech HL: Oxidase-deficient neutrophils from X-linked chronic granulomatous disease iPS cells: functional correction by zinc finger nuclease-mediated safe harbor targeting. Blood. 2011; 117: 5561-5572.

38) Hu K, Yu J, Suknuntha K, Tian S, Montgomery K, Choi KD, Stewart R, Thomson JA, Slukvin II: Efficient generation of transgene-free induced pluripotent stem cells from normal and neoplastic bone marrow and cord blood mononuclear cells. Blood. 2011; 117: e109-e119.

39) Carette JE, Pruszak J, Varadarajan M, Blomen VA, Gokhale S, Camargo FD, Wernig M, Jaenisch R, Brummelkamp TR: Generation of iPSCs from cultured human malignant cells. Blood. 2010; 115: 4039-4042.

40) Kumano K, Arai S, Hosoi M, Taoka K, Takayama N, Otsu M, Nagae G, Ueda K, Nakazaki K, Kamikubo Y, Eto K, Aburatani H, Nakauchi H, Kurokawa M: Generation of induced pluripotent stem cells from primary chronic myelogenous leukemia patient samples. Blood. 2012; 119: 6234-6242.

41) Tanaka T, Takahashi K, Yamane M, Tomida S, Nakamura S, Oshima K, Niwa A, Nishikomori R, Kambe N, Hara H, Mitsuyama M, Morone N, Heuser JE, Yamamoto T, Watanabe A, Sato-Otsubo A, Ogawa S, Asaka I, Heike T, Yamanaka S, Nakahata T, Saito MK: Induced pluripotent stem cells from CINCA syndrome patients as a model for dissecting somatic mosaicism and drug discovery. Blood. 2012; 120: 1299-1308.

42) Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, Lensch MW, Cowan C, Hochedlinger K, Daley GQ: Disease-specific induced pluripotent stem cells. Cell. 2008; 134: 877-886.

43) Batista LF, Pech MF, Zhong FL, Nguyen HN, Xie KT, Zaug AJ, Crary SM, Choi J, Sebastiano V, Cherry A,

Giri N, Wernig M, Alter BP, Cech TR, Savage SA, Reijo Pera RA, Artandi SE: Telomere shortening and loss of self-renewal in dyskeratosis congenita induced pluripotent stem cells. Nature. 2011; 474: 399-402.

44) Pessach IM, Ordovas-Montanes J, Zhang SY, Casanova JL, Giliani S, Gennery AR, Al-Herz W, Manos PD, Schlaeger TM, Park IH, Rucci F, Agarwal S, Mostoslavsky G, Daley GQ, Notarangelo LD: Induced pluripotent stem cells: a novel frontier in the study of human primary immunodeficiencies. J Allergy Clin Immunol. 2011; 127: 1400-1407.e4.

45) Seifinejad A, Taei A, Totonchi M, Vazirinasab H, Hassani SN, Aghdami N, Shahbazi E, Yazdi RS, Salekdeh GH, Baharvand H: Generation of human induced pluripotent stem cells from a Bombay individual: moving towards "universal-donor" red blood cells. Biochem Biophys Res Commun. 2010; 391: 329-334.

46) Weatherall D, Clegg J: The thalassaemia syndromes, 2nd ed. Wiley-Blackwell, New Yolk, 2001.

47) Winkelstein JA, Marino MC, Johnston RB Jr, Boyle J, Curnutte J, Gallin JI, Malech HL, Holland SM, Ochs H, Quie P, Buckley RH, Foster CB, Chanock SJ, Dickler H: Chronic granulomatous disease. Report on a national registry of 368 patients. Medicine (Baltimore). 2000; 79: 155-169.

48) Hanna J, Markoulaki S, Schorderet P, Carey BW, Beard C, Wernig M, Creyghton MP, Steine EJ, Cassady JP, Foreman R, Lengner CJ, Dausman JA, Jaenisch R: Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. Cell. 2008; 133: 250-264.

49) Saito M, Nishikomori R, Kambe N, Fujisawa A, Tanizaki H, Takeichi K, Imagawa T, Iehara T, Takada H, Matsubayashi T, Tanaka H, Kawashima H, Kawakami K, Kagami S, Okafuji I, Yoshioka T, Adachi S, Heike T, Miyachi Y, Nakahata T: Disease-associated CIAS1 mutations induce monocyte death, revealing low-level mosaicism in mutation-negative cryopyrin-associated periodic syndrome patients. Blood. 2008; 111: 2132-2141.

50) Saito M, Fujisawa A, Nishikomori R, Kambe N, Nakata-Hizume M, Yoshimoto M, Ohmori K, Okafuji I, Yoshioka T, Kusunoki T, Miyachi Y, Heike T, Nakahata T: Somatic mosaicism of CIAS1 in a patient with chronic infantile neurologic, cutaneous, articular syndrome. Arthritis Rheum. 2005; 52: 3579-3585.

51) Gore A, Li Z, Fung HL, Young JE, Agarwal S, Antosiewicz-Bourget J, Canto I, Giorgetti A, Israel MA, Kiskinis E, Lee JH, Loh YH, Manos PD, Montserrat N, Panopoulos AD, Ruiz S, Wilbert ML, Yu J, Kirkness EF, Izpisua Belmonte JC, Rossi DJ, Thomson JA, Eggan K, Daley GQ, Goldstein LS, Zhang K: Somatic coding mutations in human induced pluripotent stem cells. Nature. 2011; 471: 63-67.

52) Kim K, Doi A, Wen B, Ng K, Zhao R, Cahan P, Kim J, Aryee MJ, Ji H, Ehrlich LI, Yabuuchi A, Takeuchi A, Cunniff KC, Hongguang H, McKinney-Freeman S, Naveiras O, Yoon TJ, Irizarry RA, Jung N, Seita J, Hanna J, Murakami P, Jaenisch R, Weissleder R, Orkin SH, Weissman IL, Feinberg AP, Daley GQ: Epigenetic memory in induced pluripotent stem cells. Nature. 2010; 467: 285-290.

53) Marchetto MC, Carromeu C, Acab A, Yu D, Yeo GW, Mu Y, Chen G, Gage FH, Muotri AR: A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. Cell. 2010; 143: 527-539.

54) Mekhoubad S, Bock C, de Boer AS, Kiskinis E, Meissner A, Eggan K: Erosion of dosage compensation impacts human iPSC disease modeling. Cell Stem Cell. 2012; 10: 595-609.