Conclusions

According to the current medical technologies, after the onset of a disease, patients are diagnosed and treated. Although the significance of the prevention of chronic diseases is well recognized, preventive medicine, which has been developed based on epidemiological studies and statistics, cannot be applied to individuals, and cannot provide a precise diagnosis or individualized therapeutics. Theoretically, everybody has disease-relevant SNPs, and every person has an increased change of becomes a patient during his/her lifetime. The iPSC technology will contribute to personalized, predictive, preemptive (Zerhouni, 2005; Auffray *et al*, 2009) and precision medicine (Mirnezami *et al*, 2012).

Supplementary information for this article is available online: http://emboj.embopress.org

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Author contributions

SY: conceived this project. HI: designed the figures and the concepts. SY and HI: wrote the paper. NN and HK: collected and analyzed the data of Supplementary Table S1.

Conflict of interest

S.Y. is a member without salary of the scientific advisory boards of iPierian, iPS Academia Japan, Megakaryon Corporation and HEALIOS K. K. Japan.

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Support Information Table S1. iPSC disease modeling

Disease	Gene and Mutation	Phenotypes	Control	Reference
Neurological				
Alzheimer's disease	*APP* E603Δ *APP* V717L Sporadic	Intracellular Abeta oligomer accumulation. Increased Abeta 42/40 ratio. ER stress. Oxidative stress. Neuronal death without neurotrophic factors.	healthy iPSC	Cell Stem Cell. 2013 Apr 4;12(4):487-96.
Alzheimer's disease	*APP* duplication Sporadic	Increased Abeta40. Increased phospo-Tau (pT231). Increased active GSK-3beta.	healthy iPSC	Nature. 2012 Jan 25;482(7384):216-20.
Alzheimer's disease	*PS1* A246E *PS2* N141I	Increased Abeta 42/40 ratio.	healthy iPSC	Hum Mol Genet. 2011 Dec 1;20(23):4530-9.
Alzheimer's disease	*presenilin-1* *presenilin-2*	hiN cells from familial Alzheimer disease (FAD) patients with presenilin-1 or -2 mutations exhibit altered. Processing and localization of amyloid precursor protein (APP) and increased production of Aβ.	unaffected hiN cells	Cell. 2011 Aug 5;146(3):359-71.
Frontotemporal dimentia	*PGRN* S116X	Decreased PGRN. Activated MEK-MAPK pathway, comparing to PI3-Akt pathway.	healthy iPSCs (An age-matched subject, a clinically normal 64-year-old male with no mutations in GRN, MAPT, or C9ORF7)	Cell Rep. 2012 Oct 25;2(4):789-98.
Frontotemporal dementia	*C9ORF72* intron GGGGCC repeat expansions	GGGGCC repeats instability during neuronal differentiation. Form RNA foci. Gly-Pro dipeptide accumulation. Vulunerability to chloroquine and 3-methyladenine.	healthy iPSC	Acta Neuropathol. 2013 Sep;126(3):385-99.
Amyotrophic lateral sclerosis	*SOD1 L144F*	Sustained SOD1 mutation in iPSCs.	ESCs fibroblast from healthy control individual	Science 2008 Aug 29 321;(5893):1218-21.
Amyotrophic lateral sclerosis	*TARDBP G298S* *TARDBP M337V* *TARDBP Q343R*	Increasd TDP43 in insoble fraction short neurite length.	healthy iPSC	Sci Transl Med. 2012 Aug 1;4(145):145ra104.
Amyotrophic lateral sclerosis	*TARDBP* M337V	Increased TDP43 in soluble fraction neuronal death.	healthy iPSC	PNAS. 2012 Apr 10;109(15):5803-8.
Amyotrophic lateral sclerosis	*TARDBP* M337V	Increased TDP43 in soluble fraction astroglial cell death.	healthy iPSC	PNAS. 2013 Mar 19;110(12):4697-702.
Amyotrophic lateral sclerosis	*VAPB* P56S	Decreaed VAPB. Lack of VAPB positive inclusion. Increased VAPB instability by MG-132.	healthy iPSC ESC	Hum Mol Genet. 2011 Sep 15;20(18):3642-52.
Amyotrophic lateral sclerosis	sporadic	Increased TDP-43 aggregates. Phospho-Ser 409/410 TDP-43 aggregates.	healthy iPSC	Mol Cell Neurosci. 2013 Jul 25;56C:355-364.
Spinal muscular atrophy Parkinson disease Amyotrophic lateral sclerosis	*SMA Exon7 deletion*	n.e.	n.e.	J Reprod Dev. 2012;58(5):515-21. Epub 2012 May 24.
Spinal muscular atrophy type I	*SMA1*	Increase in Fas ligand-mediated apoptosis Increased caspase-8 and -3 activation.	unaffected iPSC	PLoS One. 2012;7(6):e39113. doi: 10.1371/journal.pone.0039113. Epub 2012 Jun 19.
Spinal muscular atrophy type I	*SMA1*	Short axon length, small growth cone, fewer and smaller motor endplates, decreased NMJ.	wild type iPSC heterozygous SMA iPSC genetically corrected patient-specific iPSC	Sci Transl Med. 2012 Dec 19;4(165):165ra162.
Spinal muscular atrophy	*SMN1*	SMA iPSC-derived astrocytes show morphological signs of activation, increased ERK1/2 activation and decreased GDNF production. SMA iPSC-derived astrocyte cultures have disrupted calcium signaling.	healthy iPSC	Glia. 2013 Sep;61(9):1418-28. doi: 10.1002/glia.22522. Epub 2013 Jul 10.
Spinal muscular atrophy	*SMN1* deletion	Reduced differentiation to motoneurons. Decreased number of gem bodies. Abnormal neurite outgrowth.	healthy iPSC	Stem Cells. 2011 Dec;29(12):2090-3.
Spinal muscular atrophy	*SMA* Exon7 deletion	Reduced differentiation to motoneurons. Decreased intranuclear GEM.	healthy iPSC	Nature. 2009 Jan 15;457(7227):277-80.
Spinal muscular atrophy	SMN1 deletion	Lack of response to VPA. Increased expression of the fatty acid translocase CD36 in VPA non-responders.	healthy iPSC ESC	Hum Mol Genet. 2013 Jan 15;22(2):398-407.
Spinobulbar muscular atrophy	*AR* CAG repeat	AR accumulation by Dihydrotestosterone.	healthy iPSC	J Biol Chem. 2013 Mar 22;288(12):8043-52.
Parkinson's disease	*LRRK2* G2019S	Decreased neurite length of dopaminergic neurons. TH positive cell death by H2O2. Increased cleaved-Caspase3 by 6-OHDA.	healthy iPSC	Cell Stem Cell. 2011 Mar 4;8(3):267-80.
Parkinson's disease	*LRRK2* G2019S Sporadic	Accumulation of alpha synuclein. Decreased neurite length of dopaminergic neurons increaed autophagy activity. Increased cleaved-Caspase3 postive neurons.	healthy iPSC	EMBO Mol Med. 2012 May;4(5):380-95.
Parkinson's disease	*LRRK2* G2019S	Decreased neurite length of dopaminergic neurons. Increased cleaved-Caspase3 by 6-OHDA or Rothenone. Increased Tua, phospho-Tau. Increased alpha-Synuclein. Increased phospho-ERK.	healthy iPSC	Cell Stem Cell. 2013 Mar 7;12(3):354-67.
Parkinson's disease	*LRRK2 G2019S*	Increased susceptibility to proteasomal stress.	healthy iPSC	Nature. 2012 Nov 22;491(7425):603-7.
Parkinson's disease	*PINK1* V170G	PARKIN translocation to mitochonoria and ubiquitination, depend on PINK1. Dysfunction of Mitophagy.	healthy iPSC	J Biol Chem. 2013 Jan 25;288(4):2223-37.
Parkinson's disease	*PINK1* Q456X *PINK1* V170G	PARKIN translocation to mitochonoria. Decreaed number of mitochondoria. Increased PGC-1alpha.	healthy iPSC	J Neurosci. 2011 Apr 20;31(16):5970-6.
Parkinson's disease	*PINK1* Q456X *LRRK2* R1441C *LRRK2* G2019S	Increaed ROS by Valinomycin. Respiratory dysfunction of mitochondria. Randomaized mitochodorial movement in Axon.	healthy iPSC	Sci Transl Med. 2012 Jul 4;4(141):141ra90.
Parkinson's disease	*PARKIN* ex2-4 deletion *PARKIN* ex6,7 deletion	Increased oxidative stress. Alpha-synuclein accmulation.	healthy iPSC	Mol Brain. 2012 Oct 6;5:35.
Parkinson's disease	*SNCA* triplication	Increased alpha-synuclein of cytosol or culture medium.	healthy iPSC	Nat Commun. 2011 Aug 23;2:440.
Parkinson's disease	*SNCA* triplication	Alpha-synuclein accmulation. Increased cleaved-Caspase3 by H2O2.	healthy iPSC	PLoS One. 2011;6(11):e26159.
Parkinson's disease	*Parkin*	Loss of parkin in human midbrain DA neurons greatly increases the transcription of monoamine oxidases and oxidative stress, significantly reduces DA uptake and increases spontaneous DA release.	healthy iPSC	Nat Commun. 2012 Feb 7;3:668
Parkinson's disease	*idiopathic*	-	non-PD iPSCs ESCs	Cell. 2009 Mar 6;136(5):964-77
Parkinson's disease	-	Mn exposure was associated with significantly higher ROS generation in NPC.	healthy iPSC	Neurotoxicology. 2012 Dec;33(6):1443-9.
Parkinson's disease	*α-Synuclein A53T (G209A)*	-	ESCs	Cell. 2011 Jul 22;146(2):318-31
Gaucher disease	*Gcase* N370S/84GG insertion	Alpha-synuclein accmulation. Increased alpha-synuclein in insoluble fraction.	healthy iPSC	Cell. 2011 Jul 8;146(1):37-52.
Gaucher disease	*GBA1*	Low acid-β-glucosidase activity.	wild-type iPSC isogenic iPSC	Hum Mol Genet. 2013 Feb 15;22(4):633-45
Gaucher disease	*GBA* N370S/N370S *GBA* L444P/RecNcil *GBA* L444P/L444P	Low glucocerebrosidase activity. Accumulated sphingolipids.	healthy iPSC ESC	Proc Natl Acad Sci U S A. 2012 Oct 30;109(44):18054-9.
Huntington disease	*HTT* CAG repeat	Neuronal death without neurotrophic factors.	healthy iPSC	PLoS Curr. 2010 Oct 28;2:RRN1193.
Huntington disease	*HTT* CAG repeat	Increased cleaved-Caspase3. Neuronal death without neurotrophic factors.	healthy iPSC	Cell Stem Cell. 2012 Aug 3;11(2):264-78.
Huntington disease	*HTT* CAG repeat	Increased cleaved-Caspase3. Neuronal death without neurotrophic factors.	healthy iPSC	Cell Stem Cell. 2012 Aug 3;11(2):253-63.
Huntington disease	*HTT CAG repeat*	Increased Susceptibility to HD Aggregate Formation in HD-iPSC	normal iPSC ESC	Stem Cells. 2012 Sep;30(9):2054-62
Huntington disease	*HTT CAG repeat*	Up-regulation of oxidative stress-related proteins in HD-iPSCs. Induction of DNA damage-mediated apoptosis in HD-iPSCs. Down-regulation of cytoskeleton-associated proteins in HD-iPSCs.	normal iPSC ESC	Biochem J. 2012 Sep 15;446(3):359-71
Huntington disease	*HTT CAG repeat*	Astrocytes generated from the HD-iPSCs with increased cytoplasmic vacuolation.	normal iPSC	Mol Brain. 2012 May 21;5:17.
Homozygous and heterozygous Huntington	*HTT CAG repeat*	Enhanced lysosomal activity.	wild type iPSC	Neurobiol Dis. 2012 Apr;46(1):41-51
Fragile X syndrome	*FMR1* CGG repeat expansion	Epigenetic modification of FMR1. Loss of FMR protein expression. Decreased neurite length.	healthy iPSC	PLoS One. 2011;6(10):e26203.
Fragile X syndrome	*FMR1*	Significant DNA methylation differences in the FMR1 promoter and 5' UTR.	iPSC (normal controls)	BMC Med Genet. 2013 Jan 29;14:18.

Fragile X-associated tremor/ataxia syndrome (FXTAS)	*FMR1*	iPS cell-derived neurons harboring the stably-active, expanded allele (EX-Xa) have reduced postsynaptic density protein 95 (PSD95) protein expression, synaptic puncta density and neurite length. EX-Xa eurons are functionally abnormal, with calcium transients of higher amplitude and increased frequency than for neurons harboring the normal-active allele. A sustained calcium elevation was found in the EX-Xa neurons after glutamate application.	iPSC derived from fibroblasts with an active normal allele	Hum Mol Genet. 2012 Sep 1;21(17):3795-805.
Familial dysautonomia	*IKBKAP* Exon20 skip	Increased abnormal splicing variant. Migration dysfunction.	healthy iPSC	Nature. 2009 Sep 17;461(7262):402-6.
Familial dysautonomia	*IKBKAP* Exon20 skip	Decreased IKBAP.	healthy iPSC	Nat Biotechnol. 2012 Dec;30(12):1244-8.
Olivopontocerebellar atrophy(OPCA)	*spinocerebellar ataxia 7 (SCA7)*	-	-	Tohoku J Exp Med. 2012;226(2):151-9.
Spinal cerebral ataxia type2	*ATXN2* hetero	Decreased ataxin-2 expression in neural stem cells or fibroblasts. *Short-lived character in Time-lapsed neural growth assay.*	healthy iPSC	J Mol Neurosci. 2013 Oct;51(2):237-48. doi: 10.1007/s12031-012-9930-2. Epub 2012 Dec 9
Spinal cerebral ataxia type3 (Machado-Joseph disease)	*ATXN3* CAG repeat	ATXN3 accumulation in insoluble faraction by glutamate stimulation.	healthy iPSC ESC	Nature. 2011 Nov 23;480(7378):543-6.
Friedreich Ataxia (FRDA)	*FXN*	GAA repeat expansion. Reduced FXN mRNA expression.	iPSC (control)	Stem Cell Rev. 2011 Sep;7(3):703-13.
Friedreich Ataxia (FRDA)	*FXN*	Repeat instability of GAA-TTC repeats in FXN. MSH2 highly expressed in pluripotent cells.	healthy iPSC SMA iPSC	Cell Stem Cell. 2010 Nov 5;7(5):631-7.
Friedreich ataxia (FRDA)	*hyperexpansion of the triplet-repeat sequence GAA.TTC within the first intron of the FXN gene*	GAA· TTC triplet-repeat expansion.	Unaffected iPSC	J Biol Chem. 2012 Aug 24;287(35):29861-72.
ataxia-telangiectasia (A-T)	*ATM*	Neural derivatives lack expression of ATM protein. Neural progenitor cells lacked substantial ATM phosphorylation caused by IR(2Gy). Cells from AT patients display decreased mitochondrial membrane potential.	wild type iPSC	Nat Commun. 2013;4:1824.
ataxia-telangiectasia (A-T)	*ATM*	Defective radiation-induced signaling, radiosensitivity, and cell cycle checkpoint defects. Abnormalities in DNA damage signaling pathways.	healthy iPSC SMA iPSC	Stem Cells Transl Med. 2012 Jul;1(7):523-35..
Dravet syndrome	*SCN1A* R1645*	Decreaed GABAergic firing, detected by patch-clamp record.	healthy iPSC	Mol Brain. 2013 May 2;6:19
Dravet syndrome	*SCN1A* F1415I SCN1A Q1923R	Increased paroxysmal deporalization shfit.	healthy iPSC	Hum Mol Genet. 2013 Jun 27
Dravet syndrome	*SCN1A*	DS patient-derived neurons show increased sodium currents in both bipolar-and pyramidal-shaped neurons.	unaffected iPSC	Ann Neurol. 2013 Jul;74(1):128-39.
Rett syndrome	*MECP2* T158M	Decreaed MeCP2. Normal differentiation to Neural precursors, but decreased number of matured TUJ1-positive neurons.	healthy iPSC ESC	Proc Natl Acad Sci U S A. 2011 Aug 23;108(34):14169-74.
Rett syndrome	*MECP2* Q244X *MECP2* R308C	Decreaesd MeCP2. Decreaed VGLUT1 puncta. Decreased cell body size. Decreaed spontanueous firing of neurons. Decreased EPSP firing.	healthy iPSC	Cell. 2010 Nov 12;143(4):527-39.
Rett syndrome	*MECP2*	Inactive X-chromosome in a onrandom pattern. RTT-hiPSC cell-derived neurons demonstrated a reduction in soma size.	isogenic iPS	Hum Mol Genet. 2011 Jun 1;20(11):2103-15.
Rett syndrome	*MECP2*	Smaller nuclear size.	isogenic iPSC	PLoS One. 2011;6(9):e25255. doi: 10.1371/journal.pone.0025255. Epub 2011 Sep 26.
HSV-1 encephalitis	*UNC93B1* c.1034_1037del4 *TLR3* c.1660C>T *TLR3* c.2236G>T	Impaired IFN-beta/lambda1 induction in dsRNA analogue or HSV1 infection.	healthy iPSC	Nature. 2012 Nov 29;491(7426):769-73.
Multiple sclerosis	Sporadic	n.e.	ESCs	Stem Cell Res. 2012 Mar;8(2):259-73.
Pelizaeus-Merzbacher disease	*a rare partial duplication of PLP1*	null PLP1 expression.	healthy iPSC	J Hum Genet. 2012 Sep;57(9):580-6.
mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS)	*ND5 subunit of complex I, G13513A*	Reduction in mitochondrial density and oxygen consumption. Consistent with the cellular mosaicism of the original patient-derived fibroblasts, the MELAS-IPSC clones contained a similar range of mtDNA heteroplasmy of the disease-causing mutation with identical profiles in the remaining mtDNA.	wild type iPSC	Stem Cells. 2013 Jul;31(7):1298-308.
Moyamoya disease (MMD)	*RNF213 R4810K polymorphism*	Angiogenic activities of iPSC-derived vascular endothelial cells (iPSECs) from patients and carriers were lower than from wild-type subjects. Gene expression profiles in iPSECs showed that Securin was down-regulated in carriers and patients.	wild type iPSC	Biochem Biophys Res Commun. 2013 Aug 16;438(1):13-9.
Glioblastoma	-	Widespread resetting of DNA methylation, but malignant cellular behavior in the neural leneage.	iPSC from the neural stem cell line CB660 (normal control) ESCs	Genes Dev. 2013 Mar 15;27(6):654-69.
childhood cerebral ALD (CCALD), adrenomyeloneuropathy (AMN)	*ABCD1*	Abnormal accumulation of VLCFA in oligodendrocytes.	wild-type iPSC	Ann Neurol. 2011 Sep;70(3):402-9.
Muscular				
Duchenne muscular dystrophy	*DMD* del exons 4–43	n.e.	genetically corrected patient-specific iPSC	Mol Ther. 2010 Feb;18(2):386-93.
Facioscapulohumeral Dystrophy (FSHD)	*deletion of a subset of the D4Z4 macrosatellite repeat*	Expression of full-length DUX4 persists in differentiated FSHD iPS cells.	control iPSC	PLoS Genet. 2010 Oct 28;6(10):e1001181.
limb-girdle muscular dystrophy 2D (LGMD2D)	*SGCA*	No SGCA expression in a differentiated myotube.	isogenic iPSC	Sci Transl Med. 2012 Jun 27;4(140):140ra89.
Myotonic dystrophy type 1 (DM1) Huntington disease	*DMPK1*	Different CTG·CAG repeat lengths among 41 iPSC clones. The correlation between repeat length and expansion rate identified the interval between 57 and 126 repeats as being an important length threshold where expansion rates dramatically increased. Longer repeats showed faster triplet-repeat expansion.	isogenic fibroblasts	Hum Mol Genet. 2013 Aug 28. doi: 10.1093/hmg/ddt386
Miyoshi myopathy	*DYSF* not discribed	Decreased expression of dysferlin. Extensive uptake of FM1-43, indicating defective membrane repair.	healthy iPSC	PLoS One. 2013 Apr 23;8(4):e61540.
Supravalvular aortic stenosis	*ELN* exon9 ins GTAT (premature termination)	Filament bundles of smooth muscle actin down. ELN down.	healthy iPSC	Circulation. 2012 Oct 2;126(14):1695-704.
Ocular				
Gyrate atrophy	*OAT* A226V	n.e.	genetically corrected patiet-specific iPSC	Proc Natl Acad Sci U S A. 2011 Apr 19;108(16):6537-42.
Retinitis pigmentosa (RP)	*RP1* 721Lfs711X *RP1* 721Lfs711X *RP9* H137L *PRPH2* W316G *RHO* G188R	8-OHdG upregulation. in vitro degeneration (Cleaved Casplase-3) up.	healthy iPSC	PLoS One. 2011 Feb 10;6(2):e17084.
Retinitis pigmentosa (RP)	*MAK (male germ cell-associated kinase)*	In the proband with the Alu insertion, the developmental switch to the MAK transcript bearing exons 9 and 12 did not occur.	healthy iPSC non-MAK-associated RP iPSC	Proc Natl Acad Sci U S A. 2011 Aug 23;108(34):E569-76.
Retinitis pigmentosa (RP)	*RHO*	In the differentiated rod cells, diffused distribution of RHO protein in cytoplasm and expressions of endoplasmic reticulum (ER) stress markers strongly indicated the involvement of ER stress. The rod cell numbers decreased significantly after successive culture.	wild type iPSC	Stem Cells Transl Med. 2012 Jun;1(6):503-9.
Autosomal recessive RP	*USH2A*	Analysis of the USH2A transcripts of these cells revealed that one of the patient's mutations causes exonification of intron 40, a translation frameshift and a premature stop codon. Western blotting revealed upregulation of GRP78 and GRP94.	hiPSC (normal)	Elife. 2013 Aug 27;2:e00824. doi: 10.7554/eLife.00824.
Best disease (BD)	*BEST1*	RPE from mutant hiPSCs displayed disrupted fluid flux and increased accrual of autofluorescent material after long-term POS feeding. RHODOPSIN degradation after POS feeding was delayed in BD hiPSC-RPE.	unaffected sibling iPSC	Hum Mol Genet. 2013 Feb 1;22(3):593-607.
Catalact	sporadic	Conexin43 up.	ESCs	PLoS One. 2012;7(3):e32612.
Haemotological				
alpha-thalassemia	*SEA, FIL*	globin chain imbalance	isogenic iPSC ESC	Blood. 2012 Nov 8;120(19):3906-14.
beta-thalassemia	*codon 41/42 4-bp(CTTT) deletion*	-	-	Proc Natl Acad Sci U S A. 2009 Jun 16;106(24):9826-30.

beta-thalassemia	*beta-thalassemia*	-	ESC	J Reprod Dev. 2012;58(4):404-9.
beta-thalassemia	*HBB*	n.e.	n.a.	Cell Res. 2009 Sep;19(9):1120-3.
beta-thalassemia	*HBB*	n.e.	ESCs	Cell Res. 2012 Apr;22(4):637-48.
beta-thalassemia	*HBB*	n.e.	n.a.	Nat Biotechnol. 2011 Jan;29(1):73-8.
Sickle cell disease	*HBB*	n.e.	isogenic iPSC	Blood. 2011 Oct 27;118(17):4599-608
Sickle cell disease	*HBB*	n.e.	isogenic iPSC	Stem Cells. 2011 Nov;29(11):1717-26.
Sickle cell disease	*HBB*	-	isogenic iPSC	Cell Res. 2011 Dec;21(12):1740-4.
Aplastic anemia hypocellular bome marrow	*TERT* *TERC*	Defective telomere elongation and hematopoiesis.	healthy iPSC	J Clin Invest. 2013 May 1;123(5):1952-63.
Fanconi Anemia	*FANCC*	Much higher frequency of chromosomal abnormalities, inability to generate teratoma composed of all three germ layers in vivo.	hiPSC/ESC	Stem Cells. 2013 May;31(5):1022-9.
Fanconi Anemia	*Fanca*	Reduction in reprogramming efficiency. Reprogramming activates the FA pathway and induces DNA damage.	iPSC (normal)	Blood. 2012 Jun 7;119(23):5449-57.
Fanconi anemia	FANCA FANCD2	Failure to relocate to UVC-radiation-induced stalled replication forks.	healthy iPSC ESC	Nature. 2009 Jul 2;460(7251):53-9.
Myeloproliferative disorders	*JAK2-V617F*	Increased erythropoiesis PV-unique gene expression pattern.	normal iPSC	Blood. 2009 Dec 24;114(27):5473-80.
Chronic myeloid leukemia	Philadelphia translocation	Reprogramming of KBM7 cell line results in escape from oncogene addiction, resistant to imanitib.	healthy iPSC	Blood. 2010 May 20;115(20):4039-42.
Chronic myeloid leukemia	Philadelphia translocation (BCR-ABL)	Hematopoietic cells from chronic myeloid leukemia were sensitive to imanitib, iPS cells were not.	healthy iPSC ESC	Blood. 2012 Jun 28;119(26):6234-42.
JMML	*PTPN11 (p.E76K)*	Increased proliferative capacity of myeloid cells. Constitutive activation of GM-CSF. Enhanced STAT5/ERK phosphorylation.	wild-type iPSC	Blood. 2013 Jun 13;121(24):4925-9.
CINCA syndrome	*NLRP3* Y570C	Increased secretion of IL-1beta.	healthy iPSC	Blood. 2012 Aug 9;120(6):1299-308.
X-linked chronic granulomatous disease	*CYBB* L153R	Lack ROS production.	healthy iPSC	Blood. 2011 May 26;117(21):5561-72.
chronic granulomatous disease	p47 phox gp91phox	Lack ROS production. Similar cytokine profile to those of the control iPSC.	healthy iPSC ESC	Stem Cells. 2012 Apr;30(4):599-611.
Congenital amegakaryocytic thrombocytopenia	*MPL* Q186X *MPL* 1499delT	Decreased megakaryopoiesis or erythropoiesis, improved by complimentary transduction of MPL. Increased FLI1 response to TPO signaling.	healthy iPSC ESC	J Clin Invest. 2013 Sep 3;123(9):3802-14.
Severe congenital neutropenia (SCN)	*ELANE*	Neutrophil maturation arrest. Little sensitivity to G-CSF.	healthy iPSC	Proc Natl Acad Sci U S A. 2013 Feb 19;110(8):3023-8.
Shwachman-Diamond syndrome (SDS)	*SDBS*	Deficits in exocrine pancreatic and hematopoietic differentiation. Enhanced aptosis. Elevated protease levels in culture supernatants.	SBDS-deficient ESCs	Cell Stem Cell. 2013 Jun 6;12(6):727-36.

Cardiac

Long QT syndrome type 1	*KCNQ1* R190Q	Prolonged action potentials and arryhthmogenic early afterdepolarizations due to reduced IKs current. Dominant-negative effect of R190Q-KCNQ1 ion channel subunits on the trafficking of wild type KCNQ1 channels to the plasma membrane.	healthy iPSC	N Engl J Med. 2010 Oct 7;363(15):1397-409.
Long QT syndrome type 2	*KCNH2* G1681A	Prolonged action potentials.	healthy iPSC	Eur Heart J. 2011 Apr;32(8):952-62.
Long QT syndrome type 2	*KCNH2* R107W	Asymptomatic carrier with LQT2 family history used to diagnose LQT2 as hiPSCcardiomyocytes showed prolonged FPD/APD.	healthy iPSC	Dis Model Mech. 2012 Mar;5(2):220-30.
Long QT syndrome type 2	*KCNH2* A614V	Prolonged action potentials due to reduced IKr current. Early afterdepolarizations and triggered arrhythmias.	healthy iPSC	Nature. 2011 Mar 10;471(7337):225-9.
Long QT syndrome type 3	-	-	-	Circ Res. 2011 Sep 30;109(8):841-7.
Long QT syndrome type 3	*SCN5A mutation (c.5287G>A; p.V1763M)*	Dominant mutant SCN5A allele gene expression significantly prolonged action potential duration or APD an increased tetrodotoxin (TTX)-sensitive late or persistent Na+ current. A positive shift of steady state inactivation and a faster recovery from inactivation.	healthy iPSC	Int J Cardiol. 2013 Oct 15;168(6):5277-86. doi: 10.1016/j.ijcard.2013.08.015. Epub 2013 Aug 15
Long QT syndrome	*The proband was found to have a de novo SCN5A LQT-3 mutation (F1473C) and a polymorphism (K897T) in KCNH2, the gene for LQT-2.*	Analysis of the biophysics and molecular pharmacology of ion channels expressed in cardiomyocytes (CMs) differentiated from these iPSCs (iPSC-CMs) demonstrates a primary LQT-3 (Na(+) channel) defect responsible for the arrhythmias not influenced by the KCNH2 polymorphism.	wild type iPSC	J Gen Physiol. 2013 Jan;141(1):61-72.
Long QT syndrome	*KCNQ1*	A markedly prolonged field potential duration (FPD) frequent severe arrhythmia only in LQTS-iPSC-derived Ebs by the IKr blocker E4031. The IKs blocker chromanol 293B did not prolong FPD in the LQTS-iPSC-derived Ebs.	healthy iPSC	Cardiovasc Res. 2012 Sep 1;95(4):419-29.
Long QT syndrome type 1 Hypertrophic cardiomyopathy Dilated cardiomyopathy	*KCNQ1* G269S *MYH7* R663H *TNNT2* R173W	Increased susceptibility to known cardiotoxic drugs as measured by action potential duration and quantification of drug-induced arrhythmias such as early afterdepolarizations and delayed afterdepolarizations.	healthy iPSC ESC	Circulation. 2013 Apr 23;127(16):1677-91.
Catecholaminergic polymorphic ventricular tachycardia type 1	*RYR2* F2483I	Elevated diastolic Ca concentrations, reduced SR Ca content, increased susceptibility to DADs and arrhythmias after catecholaminergic stimulation.	healthy iPSC ESC	Cell Physiol Biochem. 2011;28(4):579-92.
Catecholaminergic polymorphic ventricular tachycardia type 1	*RYR2* S406L	Increased susceptibility to DADs and arrhythmia. Elevated diastolic Ca2+ concentrations, a reduced SR Ca2+.	healthy iPSC	EMBO Mol Med. 2012 Mar;4(3):180-91.
Catecholaminergic polymorphic ventricular tachycardia type 1	*RYR2* M4109R	Increased susceptibility to delayed afterdepolarizations. Whole-cell [Ca2+] transient irregularities at Ca imaging.	healthy iPSC	J Am Coll Cardiol. 2012 Sep 11;60(11):990-1000.
Catecholaminergic polymorphic ventricular tachycardia type 2	*CASQ2* D307H	Increased susceptibility to delayed afterdepolarizations. Oscillatory arrhythmic prepotentials, after-contractions and diastolic [Ca2+](i) rise. More immature phenotype with less organized myofibrils and enlarged sarcoplasmic reticulum cisternae at electron microscopy analysis.	healthy iPSC	J Cell Mol Med. 2012 Mar;16(3):468-82.
Catecholaminergic polymorphic ventricular tachycardia (CPVT)	*P2328S mutation in RyR2*	Defects in Ca(2+) cycling and electrophysiology in CPVT CMs. Catecholaminergic stress led to abnormal Ca(2+) signaling and induced arrhythmias in CPVT CMs. CPVT CMs displayed reduced sarcoplasmic reticulum Ca(2+) content. Patch-clamp recordings of CPVT CMs revealed both delayed afterdepolarizations during spontaneous beating and in response to adrenaline and also early afterdepolarizations during spontaneous beating.	healthy iPSC	PLoS One. 2012;7(9):e44660. doi: 10.1371/journal.pone.0044660. Epub 2012 Sep 4.
Arrhythmogenic right ventricular dysplasia/cardiomyopathy	*PKP2* c.2484C.T	Abnormal plakoglobin nuclear translocation and decreased beta-catenin activity. Abnormal PPAR-gamma activation only in adult-like metabolic energetics. Exaggerated lipogenesis and apoptosis Ca-handling deficits.	ESCs	Nature. 2013 Feb 7;494(7435):105-10.
Arrhythmogenic right ventricular cardiomyopathy	*PKP2*	Gene expression levels of PKP2 and plakoglobin in cardiomyocytes from ARVC-iPSCs(ARVC iPSC-CM) were significantly lower. ARVC iPSC-CMs exhibited markedly reduced immunofluorescence signals when stained for PKP2 and plakoglobin. ARVC iPSC-CMs were larger and contained darker lipid droplets.	healthy iPSC	Eur Heart J. 2013 Apr;34(15):1122-33.
Timothy Syndrome	*CACNA1C* G406R	Irregular cardiac myocyte contraction and electrical activity, excess Ca influx and prolonged APD.	healthy iPSC	Nature. 2011 Mar 10;471(7337):230-4.
Timothy syndrome	*CACNA1C* G406	Increased TH expression. Increased Norepinephrine and dopamine secretion.	healthy iPSC	Nat Med. 2011 Nov 27;17(12):1657-62
Timothy syndrome	*A point mutation in the gene encoding Ca(V)1.2*	Activity-dependent dendrite retraction.	iPSC (unaffected control)	Nat Neurosci. 2013 Feb;16(2):201-9.

Dilated cardiomyopathy	*LMNA* R225X *LMNA* exon4 ins GCCA (Frame-shit change)	abnormal nuclear morphology increased senescence and apoptosis by field electric stimulation	healthy iPSC	Aging (Albany NY). 2012 Nov;4(11):803-822.
Dilated cardiomyopathy	*TNNT2* R173W	Decreased contractility and abnormal sarcomeric alpha-actinin distribution.	healthy iPSC	Sci Transl Med. 2012 Apr 18;4(130):130ra47.
Dilated cardiomyopathy	*DES*	Diffuse abnormal DES aggregations in DCM-induced-pluripotent stem cell (iPSC)-derived cardiomyocytes the diminished maximum rate of calcium ion re-uptake, slower spontaneous beating rate failure to have sustained respond to the inotropic stress induced by administration of isoproterenol.	healthy iPSC	Hum Mol Genet. 2013 Apr 1;22(7):1395-403.
Familial hypertrophic cardiomyopathy	*MYH7* R663H	Cellular enlargement and contractile arrhythmia dysregulation of Ca2+ cycling and elevation in intracellular Ca2+.	healthy iPSC *ESC*	Cell Stem Cell. 2013 Jan 3;12(1):101-13.
LEOPARD Syndrome	*PTPN11*	Large cardiomyocyte having a higher degree of sarcomeric organization and preferential localization of NFATC4 in the nucleus.	wild-type iPSC	Nature. 2010 Jun 10;465(7299):808-12.
Marfan syndrome	*FBN1*	Enhanced activation of TGFβ signaling observed in MFS cells decreased their endogenous BMP signaling exogenous BMP antagonized the enhanced TGFβ signaling in both MFS stem cells and MFSiPS cells.	wild type iPSC ESC	Stem Cells. 2012 Dec;30(12):2709-19.
Marfan syndrome	*FBN1*	Osteogenic differentiation of ESCs with a FBN1 mutation is inhibited. Chondrogenesis is not perturbated and occurs in a TGF-β cell-autonomous fashion. Skeletal phenotypes observed in hESCs carrying the monogenic FBN1 mutation (MFS cells) are faithfully phenocopied by cells differentiated from iPSCs derived independently from MFS patient fibroblasts.	wild type iPSC	Proc Natl Acad Sci U S A. 2012 Jan 3;109(1):215-20.
Metabolic				
Wilson's disease	*ATP7B* M769V	n.e.	healthy iPSC	Protein Cell. 2012 Nov;3(11):855-63.
Wilson's disease	*ATP7B*	Abnormal cytoplasmic localization of mutated ATP7B and defective copper transport in hepatocyte-like cells from Wilson's disease.	IMR90 iPSC ESC	Hum Mol Genet. 2011 Aug 15;20(16):3176-87.
1) Alpha1-antitrypsin deficiency 2) Familial hypercholesterolemia 3) Glycogen storage disease type 1a	1) *A1AT* E342L 2) *LDL-R* n.a. 3) *G6PC*	1) increased polymeric alpha1-antitrypsin 2) decreased LDL-uptake 3) glycogen accumulation	healthy iPSC	J Clin Invest. 2010 Sep;120(9):3127-36.
Mucopolysaccharidosis type IIIB	*NAGLU* R482W	Accumulated heparan sulfate and proteoglycans. Golgi complex alterations.	healthy iPSC	Hum Mol Genet. 2011 Sep 15;20(18):3653-66.
Pompe disease	*GAA*	Low GAA activity and high glycogen content. Multiple ultrastructural aberrances.	healthy iPSC ESC	Hum Mol Genet. 2011 Dec 15;20(24):4851-64.
Lesch-Nyhan syndrome	*HPRT1* not described	Erosion of X chromosome inactivation after long time passage.	healthy iPSC	Cell Stem Cell. 2012 May 4;10(5):595-609.
Type 1 and type 2 diabetes	-	-	-	Stem Cells Transl Med. 2012 Jun;1(6):451-61.
Type 1 diabetes	-	-	-	Proc Natl Acad Sci U S A. 2009 Sep 15;106(37):15768-73.
Type 1 diabetes (T1D)	-	Notable intrapatient variation was evident upon further guided differentiation into HNF4α- and HNF1β-expressing primitive gut tube, and INS- and glucagon (GCG)-expressing islet-like cells.	nondiabetic iPSC	Mol Ther. 2013 Jan;21(1):228-39.
Type 2 diabetes (T2D)	-	Derived iPS clones acquired a rejuvenated state, characterized by elongated telomeres and suppressed senescence-related p15INK4b/p16INK4a gene expression and oxidative stress signaling.	iPSCs from non-diabetic patients	Aging (Albany NY). 2012 Jan;4(1):60-73.
Maturity onset diabetes of the young (MODY)	*HNF4A, GCK, HNF1A, HNF1B, CEL*	-	healthy iPSC	J Biol Chem. 2013 Feb 22;288(8):5353-6.
Maturity-onset diabetes of the young type 2 (MODY2)	*GCK*	β cells required higher glucose levels to stimulate insulin secretion.	healthy +A152:D154iPSC	J Clin Invest. 2013 Jul 1;123(7):3146-53.
Mitochondrial diabetes	*mtDNA A3243G mutation*	About half of the clones had undetectable levels of the mutation.	-	Diabetologia. 2012 Jun;55(6):1689-98.
familial hypercholesterolemia (FH)	*LDLR*	Inability of uptake labeled LDL particles.	genetically corrected patient-specific iPSC	Mol Biotechnol. 2013 Jul;54(3):863-73.
familial hypercholesterolemia (FH)	*LDLR*	iPSC-derived hepatocytes show deficiencies in uptake of LDL-C and in their response to lovastatin and a marked elevation in secretion of lipidated apolipoprotein B-100.	iPSC ESC	Hepatology. 2012 Dec;56(6):2163-71.
Fabry disease	*mutations in the retinal pigment epithelium (RPE) gene BESTROPHIN1*	massive membranous cytoplasmic bodies	-	Mol Genet Metab. 2013 Aug;109(4):386-9.
Mucopolysaccharidosis type I (Hurler Syndrome)	*IDUA(Y167X, W402X)*	accumulation of GAG	genetically corrected patient-specific iPSC	Blood. 2011 Jan 20;117(3):839-47.
1)Tyrosinemia type I 2)Glycogen straoge type Ib 3)Progressive familial hereditary cholestasis 4)Crigler-Najjar Syndrome	1)*FAH* Q64H 2)*SLC37A* c.1124-2A>G 3)Multifactorial 4)*UGT1A1* L413P	n.e.	n.e.	Stem Cell Rev. 2010 Dec;6(4):622-32.
Imprinting				
Angelman syndrome Pradder-Willi syndrome	*UBE3A* (15q11-q13) deletion	No evidence of DNA methylation imprint erasure at the cis-acting PSW imprinting center. UBE3A paternalimprinting re-established during hiPSC neuronal differentiation.	healthy iPSC	Proc Natl Acad Sci U S A. 2010 Oct 12;107(41):17668-73.
Pradder-Willi syndrome	*UBE3A* (15q11-q13) deletion	iPSCs retain a high level of DNA methylation in the imprinting center of the maternal allele and show concomitant reduced expression of the disease-associated small nucleolar RNA HBII-85/SNORD116.	healthy iPSC	J Biol Chem. 2010 Dec 17;285(51):40303-11.
Skin				
Recessive dystrophic epidermolysis bullosa (RDEB)	*COL7A1* E2059G *COL7A1* G1640fsX70/R2751fsX20 *COL7A1* R261X	No Col7 was detected in the skin-like structures derived from RDEB iPS cells.	healthy iPSC	J Invest Dermatol. 2011 Apr;131(4):848-56.
Recessive dystrophic epidermolysis bullosa (RDEB)	*COL7A1*	No expression of Type VII collagen.	healthy iPSC	Proc Natl Acad Sci U S A. 2011 May 24;108(21):8797-802.
Dyskeratosis congenita	*TERT* P704S *TERT* R979W DKC1 L54V TCAB1 H*Y/G*R	Disrupts telomere elongation during reprogramming telomerase mislocalizes from Cajal bodies to nucleoli and decreased telomerase activity.	healthy iPSC	Nature. 2011 May 22;474(7351):399-402.
Dyskeratosis Congenita	*DKC1*	Telomere elongation, upregulation of TERC and DKC1.	wild-type iPSC	Nature. 2010 Mar 11;464(7286):292-6.
Hermansky-Pudlak syndrome Chediak-Higashi syndrome	*HP1* exon15 16bp dupl. *HP2* G2028T *LYST* Codon40 GCA to GGCA	Decreased melanin content melanosome abnormality.	healthy iPSC	Cell Rep. 2013 Apr 25;3(4):1140-52.
other				
Down syndrome (trisomy 21)	Chr.21 trisomy	Increased Abeta 40. Fibrillar Abeta accmulation. Increased phospo-Tau (pT231, pS396).	healthy iPSC	Sci Transl Med. 2012 Mar 7;4(124):124ra29.
Down syndrome (trisomy 21)	Chr.21 trisomy	The derived, disomic cells proliferated faster and produced more endothelia in vivo than their otherwise isogenic trisomic counterparts, but in vitro hematopoietic differentiation was not consistently altered.	isogenic disomic iPSC	Cell Stem Cell. 2012 Nov 2;11(5):615-9.
Down syndrome (trisomy 21)	Chr.21 trisomy	Yielded Ts21 iPSCs and an isogenic control that is disomic for human chromosome 21 (HSA21). Ts21 neurons displayed reduced synaptic activity.	isogenic disomic iPSC	Proc Natl Acad Sci U S A. 2013 Jun 11;110(24):9962-7.
Down syndrome (trisomy 21)	Chr.21 trisomy	The level of amyloid precursor protein was significantly increased in NPCs derived from T21 AF-iPS cells. The expression levels of miR-155 and miR-802 in T21 AF-iPS-NPCs were highly elevated in the presence of low expression of MeCP2. T21 iPS-NPCs generated fewer neurons and exhibit developmental defects during neurogenesis.	normal iPSC	Exp Cell Res. 2013 Feb 15;319(4):498-505.
Down syndrome (trisomy 21)	Chr.21 trisomy	Blood progenitor populations generated from T21 iPSCs were present at normal frequency and proliferated normally. However, their developmental potential was altered with enhanced erythropoiesis and reduced myelopoiesis, but normal megakaryocyte production	euploid iPSC	Proc Natl Acad Sci U S A. 2012 Oct 23;109(43):17573-8.
Down syndrome (trisomy 21)	Chr.21 trisomy	-	-	Stem Cell Res Ther. 2012 Apr 18;3(2):14.

Down syndrome (trisomy 21)	Chr.21 trisomy	Trisomic cells of hES, iPS, or isogenic origins exhibited a two- to fivefold increase in a population of CD43(+)(Leukosialin)/CD235(+)(Glycophorin A) hematopoietic cells.	isogenic iPSC	Proc Natl Acad Sci U S A. 2012 Oct 23;109(43):17567-72.
Turner syndrome(monosomy X) trisomy 8 (Warkany syndrome 2) trisomy9 trisomy 13 (Patau syndrome) partial trisomy 11;22 (Emanuel syndrome)	-	TS iPSCs displayed lower levels of the pseudoautosomal genes ASMTL and PPP2R3B, and displayed insufficient up-regulation of the pseudoautosomal placental gene CSF2RA during embryoid body formation.	IMR90 iPSC ESC	Hum Mol Genet. 2012 Jan 1;21(1):32-45.
Klinefelter's Syndrome	*47, XXY*	Aberrantly expressed genes associated with the clinical features of KS.	normal iPSC	J Biol Chem. 2012 Nov 9;287(46):38970-9.
Atypical Werner Syndrome (AWS) Hutchinson Gilford Progeria Syndrome (HGPS) Dilated Cardiomyopathy (DCM)	*lamin A/C*	low expression of lamin A/C in iPSC nuclear abnormality, increased senescence and susceptibility to apoptosis in iPSC-derived fibroblasts	normal hiPSC	Aging (Albany NY). 2011 Apr;3(4):380-90.
Hutchinson Gilford Progeria Syndrome (HGPS)	*LMNA* c.1824C>T (splice alterlation)	Increased DNA damage and cell death at hypoxic condition. Transplantation effect for Ischemic Murine Hind Limb were decreased.	healthy iPSC ESC	Proc Natl Acad Sci U S A. 2010 Oct 12;107(41):17668-73.G181
Hutchinson Gilford Progeria Syndrome (HGPS)	*LMNA*	Absence of progerin. Lack of the nuclear envelope. Epigenetic alterations normally associated with premature ageing.	wid type iPSC	Nature. 2011 Apr 14;472(7342):221-5.
Hutchinson Gilford Progeria Syndrome (HGPS)	*LMNA*	Increased gene expression of progerin.	healthy iPSC genetically corrected patient-specific iPSC	Cell Stem Cell. 2011 Jun 3;8(6):688-94.
Cockayne syndrome (CS)	*ERCC6*	Elevated cell death rate and higher reactive oxygen species (ROS) production. Up-regulation of TXNIP and TP53 transcriptional expression.	wild type iPSC	Hum Mol Genet. 2012 Sep 1;21(17):3825-34.
Schizophrenia	-	Perturbations in neural differentiation and mitochondrial function.	healthy iPSC	Mol Psychiatry. 2013 Oct;18(10):1067-76.
Schizophrenia	Sporadic	Decreaed synaptic junction. Decreaed neurite length. Decreaed number of synapse. Spontaneous action potential or EPSP firing ratio were unchanged.	healthy iPSC	Nature. 2011 May 12;473(7346):221-5.
Schizophrenia, PD	-	Disease-specific alterations in gene expression, protein epression and cell function.	healthy ONS(olfactory neurosphere-derived) cells	Dis Model Mech. 2010 Nov-Dec;3(11-12):785-98.
autism spectrum disorder	-	-	-	Neurosci Lett. 2012 May 10;516(1):9-14.
Cystic fibrosis α-1 antitrypsin deficiency-related emphysema scleroderma sickle-cell disease	-	-	ESCs	Stem Cells. 2010 Oct;28(10):1728-40.
α1 antitrypsin deficiency	*A1AT, also known as SERPINA1*	Polymeric A1AT protein in hepatocyte-like cells decreased A1AT enzymatic inhibitory activity.	genetically corrected patient-specific iPSC	Nature. 2011 Oct 12;478(7369):391-4.
α1 antitrypsin deficiency	*A1AT*	-		Blood. 2011 Aug 18;118(7):1801-5.
ectrodactyly, ectodermal dysplasia, and cleft lip/palate (EEC) syndrome	*p63*	Early ectodermal commitment into K18(+) cells but failed to further differentiate into K14(+) cells (epidermis/limbus) or K3/K12(+) cells (corneal epithelium).	healthy iPSC	Proc Natl Acad Sci U S A. 2013 Feb 5;110(6):2152-6.
Fibrodysplasia ossificans progressiva (FOP)	*activin receptor-like kinase 2 (ALK2)*	Generation of induced pluripotent stem cells (iPSCs) from FOP-derived skin fibroblasts is repressed.	healthy iPSC	Stem Cells. 2012 Nov;30(11):2437-49.
primary immunodeficiencies	*RAG1* *STAT1* *TLR3* *RMRP*	-	healthy iPSC	J Allergy Clin Immunol. 2011 Jun;127(6):1400-7.e4.
XLF/Cernunnos syndrome	*XLF(homozygous R57X)*	Alterd in vitro differentiation capacity and inability to generate teratomas comprised of all three layers in vitro.	unaffected iPSC ESC	Stem Cells. 2013 Jul 2. doi: 10.1002/stem.1456.
familial Alzheimer's disease familial Parkinson disease	*PARK4*	increased Aβ42/40, α-synuclein	centenarian donors	PLoS One. 2012;7(7):e41572. doi: 10.1371/journal.pone.0041572. Epub 2012 Jul 25.
Alzheimer's disease Parkinson's disease juvenile-onset, type I diabetes mellitus Duchenne type muscular dystrophy	-	n.e.	n.e.	Exp Mol Med. 2012 Mar 31;44(3):202-13.
1)ADA-SCID 2)Gaucher disease type III 3)Duchenne muscular dystrophy 4)Becker muscular dystrophy 5)Down Syndrome 6)Parkinson disease 7)Juvenile diabetes mellitus 8)Shwachman-Bodian-Diamond syndrome 9)Huntington disease 10)Lesch-Nyhan Syndrome (carrier)	1)*ADA* exon7 GGG>AGG/exon10 Del(GAAGA) 2)*GBA* exon9 AAC>AGC/84G insertion 3)*dystrophin* exon45-52 deletion 4)*dystrophin* unidentified 5)Chr.21 trisomy 6)sporadic 7)sporadic 8)*SBDS* IV2+2T>C/IVS3-1G>A 9)*HTT* CAG repeat 10)*HPRT1* Heterozygosity	n.e.	n.e.	Cell. 2008 Sep 5;134(5):877-86.
ADPKD SLE Wilms tumor	*W3842X mutation in exon 41 of PKD1 gene*	-	-	Stem Cell Res Ther. 2011 Dec 6;2(6):48.
HIV	-	HIV-1 resistant and functional macrophage	healthy iPSC	Mol Ther. 2011 Mar;19(3):584-93.

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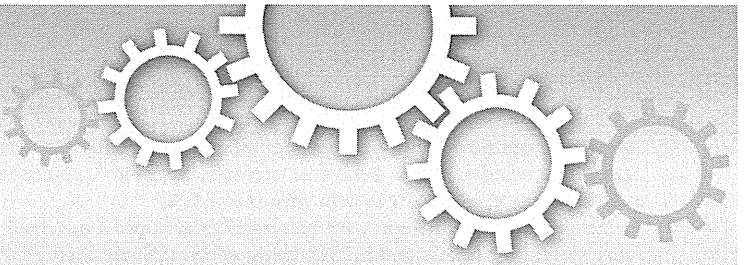

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Correspondence and requests for materials should be addressed to M.N. (nakagawa-g@cira.kyoto-u.ac.jp)

A novel efficient feeder-free culture system for the derivation of human induced pluripotent stem cells

Masato Nakagawa[1], Yukimasa Taniguchi[2], Sho Senda[3], Nanako Takizawa[1], Tomoko Ichisaka[1], Kanako Asano[1], Asuka Morizane[1], Daisuke Doi[1], Jun Takahashi[1], Masatoshi Nishizawa[1], Yoshinori Yoshida[1], Taro Toyoda[1], Kenji Osafune[1], Kiyotoshi Sekiguchi[2] & Shinya Yamanaka[1,4]

[1]Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto 606-8507, Japan, [2]Institute for Protein Research, Osaka University, Osaka 565-0871, Japan, [3]Institute for Innovation, Ajinomoto CO., Inc., Kawasaki, 210-8681 Japan, [4]Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158 USA.

In order to apply human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) to regenerative medicine, the cells should be produced under restricted conditions conforming to GMP guidelines. Since the conventional culture system has some issues that need to be addressed to achieve this goal, we developed a novel culture system. We found that recombinant laminin-511 E8 fragments are useful matrices for maintaining hESCs and hiPSCs when used in combination with a completely xeno-free (Xf) medium, StemFit™. Using this system, hESCs and hiPSCs can be easily and stably passaged by dissociating the cells into single cells for long periods, without any karyotype abnormalities. Human iPSCs could be generated under feeder-free (Ff) and Xf culture systems from human primary fibroblasts and blood cells, and they possessed differentiation abilities. These results indicate that hiPSCs can be generated and maintained under this novel Ff and Xf culture system.

Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) hold promise as tools for regenerative medicine. Recently, several reports have discussed the potential use of stem cells in clinical applications. Geron has initiated treatment of neural disease using neuronal cells derived from hESCs. Advanced Cell Technologies is making efforts to treat eye diseases with ESC-derived cells[1]. This approach involves the production of retinal pigment epithelium from hESCs that are then transplanted into patients. Regenerative medicine using stem cells, particularly pluripotent stem cells, will certainly advance over the coming years as new discoveries are made.

Researchers usually use feeder cells and serum-containing medium in conventional culture systems for hESCs and hiPSCs[2,3]. Murine-derived feeder cells are widely used to maintain hESCs and hiPSCs. Human-derived feeder cells are also used for hESC/iPSC culture; however, in some cases, these cells have proven unsuitable for stem cell maintenance[4,5]. The feeder cell preparation requires significant time and effort. Fetal bovine serum (FBS)-containing medium is normally used for the culture of feeder cells. The reduction or complete removal of serum and animal-derived products is required to satisfy Standard for Biological Ingredients. Moving towards feeder-free culture systems for hESCs and hiPSCs would represent a significant improvement over conventional culture systems.

To address these issues, we sought to develop a novel culture system applicable for human stem cell maintenance and hiPSC derivation. Feeder-free (Ff) and xeno-free (Xf) conditions appear to be acceptable for culturing hESCs and hiPSCs. Various matrices can be used to replace feeder cells, such as Matrigel[6–8], CELLstart[9,10], recombinant proteins[11–13] and synthetic polymers[14,15]. Xeno-free media are also available commercially, including TeSR2, NutriStem and Essential E8 medium[13], among others. Although we examined most of these materials with respect to whether the hESCs and hiPSCs could be stably and efficiently cultivated in our laboratory, we were unable to identify an efficacious combination of matrix and medium.

It has previously been reported that laminin-511 supports the stable culture of hESCs and hiPSCs[11]. Recently, a shorter fragment of laminin-511, referred to as the laminin-511 E8 fragment (LN511E8), was also shown to efficiently maintain hESCs and hiPSCs[12]. Recombinantly expressed LN511E8 (rLN511E8) is isolated more easily, and with a greater yield and purity, than full-length laminin-511. For these reasons, we chose rLN511E8 as a

matrix for our novel culture system for hESCs and hiPSCs. Next, we examined whether a new xeno-free medium, StemFit™, could be used for our novel culture system with rLN511E8.

Employing these materials, we successfully developed a novel culture system for hESCs and hiPSCs using rLN511E8 and StemFit™ that is easy to use, expandable and reproducible, as clinical-grade hiPSCs must be manufactured according to Standard Operating Procedures (SOPs) in order to meet Cell Processing Center (CPC) standards.

Human ESCs and iPSCs were stably passaged for long periods by dissociating the cells into single cells. Moreover, hiPSCs were successfully established from primary fibroblasts, peripheral blood and cord blood under these conditions using episomal vectors[16,17]. These Ff-hiPSCs displayed the capacity to differentiate into various types of somatic cells, including all three germ layers. These results indicate that Ff-hiPSCs are suitable for manufacturing in a CPC setting, and should prove useful for future research and clinical applications.

Results

Development of a novel culture system for hiPSCs. To develop feeder-free (Ff) and xeno-free (Xf) hiPSC culture conditions, we tested Matrigel, CellStart and the recombinant laminin-511 E8 fragment (rLN511E8) as coating matrices[12]. H9 hESCs were dissociated into single cells and plated onto the coated culture plates. The hESCs efficiently formed colonies on rLN511E8 but not on the other matrices (Figure S1A). We therefore selected rLN511E8 as the coating matrix for our system. Using rLN511E8, we attempted to cultivate hiPSCs using a variety of commercially available Xf-medium (Figure S1B). TeSR2 did not support the maintenance of hiPSCs (32R1[18]) on rLN511E8. When we used NutriStem, the hiPSCs formed flattened colonies. Although the mixture of TeSR2 and NutriStem supported hESC-like colony formation, the morphology was not good (many granules were detected in cells). Since we were unable to obtain good results, we chose to try StemFit™, a newly developed Xf-medium for hiPSC culture from Ajinomoto Co., Inc. Using StemFit™, we obtained hiPSCs colonies similar to those cultivated on feeder cells[2] (Figure S1B).

We examined whether hESCs and hiPSCs, which were previously established and maintained on feeder cells, can be cultivated under the Ff and Xf conditions using rLN511E8 matrix and StemFit™ (Figure 1A). After two or three passages, most of the hESCs and hiPSCs adapted to the Ff and Xf culture conditions. The combination of rLN511E8 and StemFit™ demonstrated efficacy for the hESCs and hiPSC culture.

Human iPSCs were then dissociated into single cells and reproducibly plated according to the exact cell number (Figure S2A), an important consideration for standardizing culture conditions and developing a reliable experimental design. The cells cultured on rLN-511E8 became confluent within 8–10 days after plating (the average fold change was 132 in each passage (Figure S2B)). The average doubling time was 28.34 hours (Figure S2B). This period was faster than that of hiPSCs cultivated on feeder cells[2]. Surprisingly, the high cell viability permitted a split ratio of nearly 1:100 (Figure S2B). Frozen stocks were prepared at −80°C using a standard slow-freezing method, and were thawed in a 37°C water bath (Figure S2A).

We next examined whether the hES/iPSCs could be stably cultivated over long periods using the new culture conditions. We used H9 hESCs, KhES1 hESCs and 201B7 hiPSCs for this experiment. The cells were stably maintained for 20–30 passages, and markers of pluripotency, such as Oct3/4 and TRA-1-60, were still detected (Figures 1B and 1C). Passage number 54 of the 201B7 cells still exhibited the ability to differentiate into all three germ layers *in vitro* (Figure S2C). We concluded that our method is sufficient and efficient for hESC and hiPSC culture. This culture system has already been tested and showed similar results at several other laboratories in Japan.

Establishment of hiPSCs under Ff and Xf conditions. Although we successfully developed a highly efficient system for the culture of hESCs and hiPSCs using rLN511E8 and StemFit™, hiPSCs intended for clinical application should be generated under similar conditions. First, we attempted to establish hiPSCs from human primary fibroblasts. Skin tissues were collected using biopsies, and fibroblasts were generated from the skin tissues. Fibroblasts were established under Xf conditions with medium containing 10% autologous serum. The fibroblasts were electroporated with episomal vectors containing reprogramming factors. Twenty to thirty days after electroporation, hiPSC colonies were observed and selected to establish feeder-free hiPSC (Ff-hiPSC) clones, 987A3 and 987A7 (Figure 2A). The morphology of the fibroblast-derived Ff-hiPSCs was similar to that of 201B7 or H9 cells cultivated on rLN511E8. The loss of episomal vectors was confirmed using a genomic PCR analysis (Figure S3A).

The expression levels of markers of pluripotency were examined using RT-PCR and immunostaining. The fibroblast-derived Ff-hiPSC clones exhibited similar expression levels to those of 201B7 and H9 cells (Figures 2B and S3B). The expression levels of genes related to pluripotency were similar in the cells cultivated on rLN511E8 and feeder cells (Figure 2B). The fibroblast-derived Ff-hiPSCs were stably passaged for long periods (Figure 2C) and had normal karyotypes (Figure S3C).

Ff-hiPSCs were also established from peripheral blood-derived T-cells, non-T-cells and cord blood. The morphology, marker gene expression levels and stability for long-term culture of these cells were similar to those of fibroblast-derived Ff-hiPSCs (Figures 2 and S3D).

The efficiency of Ff-hiPSC generation is summarized in Supplemental Table 1.

Differentiation capacity of Ff-hiPSCs. We examined whether Ff-hiPSCs have the ability to differentiate into several types of somatic cells. First, Ff-hiPSCs were cultivated on rLN511E8 with StemFit™ in the absence of bFGF. The cells efficiently attached to rLN511E8-coated plates and grew, exhibiting spontaneous differentiation. Two weeks after differentiation, we confirmed the expression levels of Sox17, α-smooth muscle actin (SMA) and βIII tubulin by immunostaining (Figure S4). Ff-hiPSCs were able to differentiate into all three germ layers *in vitro*. Moreover, in the teratoma assays, Ff-hiPSCs differentiated into various tissues of the three germ layers, including gut-like epithelial tissue, cartilage and neural tissue (Figure 3A). Another three Ff-hiPSC clones were also tested for the teratoma assays. These results demonstrated that the Ff-hiPSCs are functionally equivalent to iPSCs derived under feeder conditions[2], and have the potential to spontaneously differentiate into all three germ layers both *in vitro* and *in vivo*.

The directed differentiation of hiPSCs has the potential to generate various somatic cells for disease modeling, drug discovery, toxicology, prediction of side effects, and eventually, transplantation therapy. Therefore, we next examined whether Ff-hiPSCs could be specifically induced to differentiate towards somatic cells of therapeutic interest.

Parkinson's disease is characterized by the loss of dopaminergic (DA) neurons; therefore, hiPSC-derived DA neurons may be good sources for cell transplant therapy. Mature and functional DA neurons have been produced through long-term culture of hiPSCs on Matrigel[19]. We subjected Ff-hiPSCs to the neuronal differentiation protocol with dual SMAD inhibition. Consequently, the Ff-hiPSCs differentiated into DA neurons expressing tyrosine hydroxylase (TH), βIII tubulin (Tuj1), Nurr1 and Foxa2 (Figure 3B). This experiment was performed under Xf conditions. Therefore, these results indicate that DA neurons can be successfully generated under Xf conditions from human tissue samples obtained using Ff-hiPSCs cultured without Matrigel.

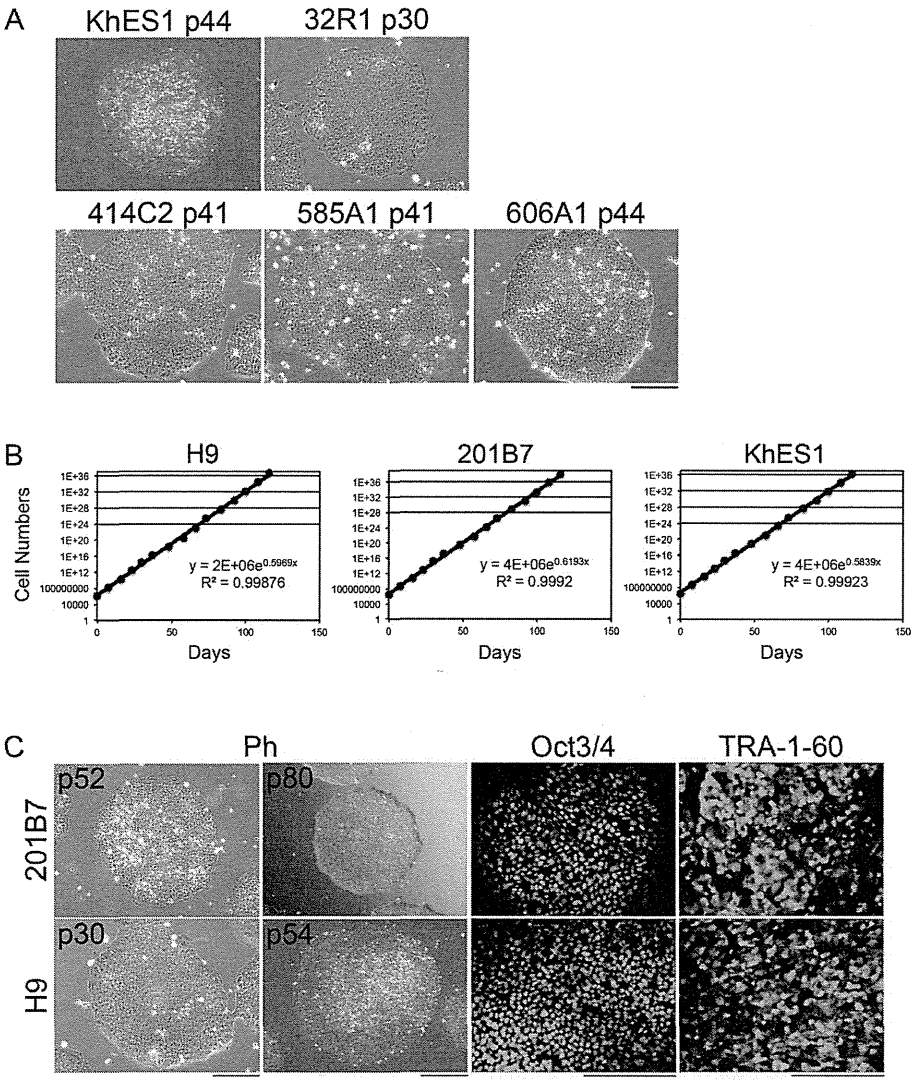

Figure 1 | The feeder-free (Ff) and xeno-free (Xf) culture system for hES/iPS cells. (A) The morphology of hESCs (KhES1) and hiPSCs (32R1, 414C2[31], 585A1[31] and 606A1[31]) cultivated on rLN511E8-coated cell culture plates with StemFit™. Scale bar, 100 μm. (B) The growth curves of the hESCs (H9 and KhES1) and hiPSCs (201B7) cultured under Ff and Xf conditions. Each dot represents a passage of cells. (C) The morphology of the indicated passage numbers of 201B7 and H9 cells. The 201B7 and H9 cells at passage numbers p80 and p54, respectively, were immunostained for the indicated pluripotency markers, followed by phase contrast imaging (Ph). Scale bar, 100 μm.

As a second target lineage with therapeutic potential, we chose to differentiate Ff-hiPSC into blood cells. Ff-hiPSCs were cultivated in a low-binding cell culture plate to promote the formation of EB-like spheres, which were sequentially treated with cytokines to invoke blood cell differentiation, as described previously[20]. Erythroblasts, macrophages and myeloid lineage cells were produced from Ff-hiPSCs, as demonstrated using May-Grunwald-Giemsa staining (Figure 3C).

The third target cells induced were insulin-producing cells. The differentiation of Ff-hiPSCs into insulin-producing cells has been reported previously[21], and we made use of a similar protocol. Consequently, insulin-producing cells were generated from Ff-hiPSCs (Figure 3D). These results indicate that Ff-hiPSCs cultured under Xf conditions have the ability to differentiate into specific cells of interest using established *in vitro* induction protocols, with some minor modifications to maintain the Xf conditions. The efficiency and quality should be examined by the future experiments.

Discussion

We developed a novel efficient culture system for hES/iPSCs without feeder cells. Recombinant LN511E8 strongly supported hESC and hiPSC culture for long periods. StemFit™, a newly developed Xf-medium, was the best medium for hESC and hiPSC culture with rLN511E8. Under this novel culture system, hESCs and hiPSCs were passaged by dissociating them into single cells. Moreover, hESC and hiPSCs could be cryopreserved at −80°C by the slow-freezing method. Ff-hiPSCs showed the ability to differentiate into several somatic cell types, similar to conventional hiPSCs cultured on feeder cells[2].

The culture system using rLN511E8 has been reported previously by Miyazaki et al.[12]. The authors also passaged hES/iPSCs by dissociating into single cells. Despite the basic idea is the same, we could develop more efficient method using StemFit™, achieving significantly better attachment efficiency at 6 hours after plating (Supplementary Table 2). Moreover, we confirmed the requirement of the glutamic acid residue in the C-terminal tail of the laminin γ1

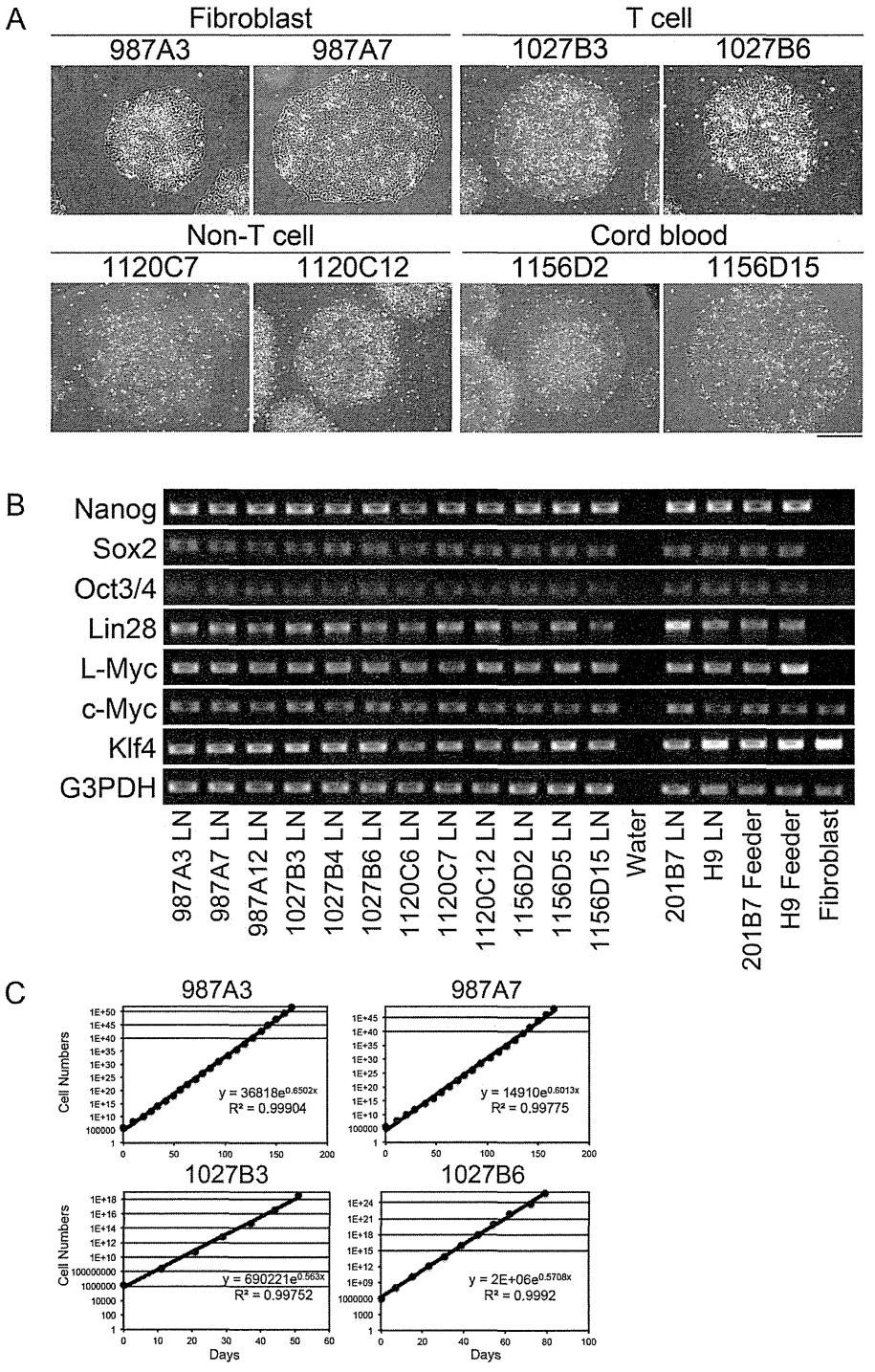

Figure 2 | Establishment of iPS cell clones under the feeder-free (Ff) and xeno-free (Xf) culture system. (A) Human iPSCs established from skin-derived primary fibroblasts (987A3 and 987A7), peripheral blood-derived T-cells (1027B3 and 1027B6), peripheral blood-derived non-T-cells (1120C7 and 1120C12) and cord blood cells (1156D2 and 1156D15) under the Ff and Xf culture system. Photographs were taken between passages p5 and p10. Scale bar, 100 μm. (B) The gene expression levels of pluripotency markers as determined using RT-PCR. "LN" indicates that the cells were cultivated using the Ff and Xf culture system reported herein. "Feeder" indicates cells that were cultivated on murine feeders with non-Xf medium. (C) The growth curve of the feeder-free iPS cells cultured under Ff and Xf culture conditions.

chain, substitution of which with glutamine abolishes the integrin binding activity of LN511E8[24], in our culture method using an EQ mutant of rLN511E8. The colony formation of 201B7 was not observed on EQ mutant-coated plate (Figure S5).

Recently, the defined and simplified Xf medium, E8, was reported[13]. The major difference in formulation between E8 and StemFit™ is albumin. StemFit™ includes human albumin (E8 does not include any albumins). Albumin has many biological and

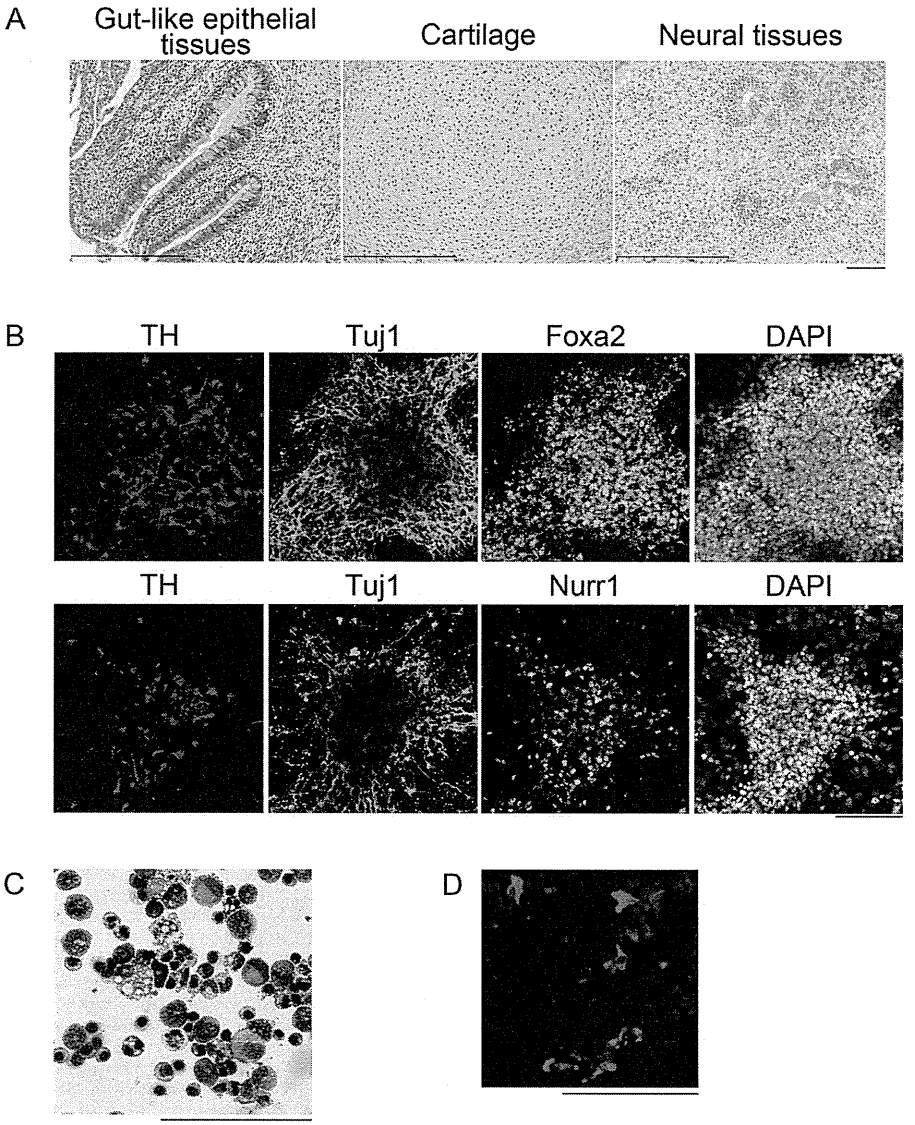

Figure 3 | The differentiation capacity of the T-cell-derived feeder-free hiPSC clone, 1027B6 (p7–p12). (A) Hematoxylin and eosin staining of teratomas showing representative derivatives of all three germ layers. Scale bar, 100 μm. (B) Differentiation into mesencephalic dopaminergic neurons under Xf conditions. Photomicrographic images of immunostaining for Tuj1 (green), tyrosine hydroxylase (TH: red), Foxa2 or Nurr1 (blue) and DAPI (white). Scale bar, 100 μm. (C) May-Grünwald-Giemsa staining of differentiated blood cells on day 16 showing hematopoietic precursor cells, myeloid precursor cells, macrophages and erythroblasts. Scale bar, 100 μm. (D) Feeder-free hiPSCs differentiated into insulin-producing cells. After 23 days of culture under the differentiation conditions, the cells were fixed and stained with Hoechst33342 (blue) and anti-insulin antibodies (magenta). Scale bar, 100 μm.

physical roles. Thus, this factor may confer difference in performance between the both.

We are planning to build up a bank of hiPSCs for transplantation therapy. The human leukocyte antigen (HLA) is a key factor that mediates the immune-related rejection after transplantation. To minimize the immune system-related rejection, it is necessary to match the HLA type of the donor and recipient. Matching the HLA type is difficult because of the large number of HLA types present in each individual. However, the Japanese population is relatively homogeneous compared to other populations, and it has been reported that 50–140 HLA-homozygous cell lines would match 90% of the Japanese population[17,22], a HLA-homozygous hiPSC bank would therefore be a helpful resource for therapeutic application in Japan.

Ensuring the quality and safety of hiPSC are important for their clinical application. Manufacturing hiPSCs should be performed in the Cell Processing Center under the GMP guidelines. Our novel hiPSC culture system is comparable to that of standard cell lines, such as 293 cells or HeLa cells, making previously complex steps more routine. Employing this easy to use, reproducible and expandable culture system, a large amount of clinical-grade hiPSC stock can be made at early passage numbers at the same time. Moreover, the procedures needed to establish and maintain Ff-hiPSCs should be minimal and simple in the CPC. In order to apply Ff-hiPSCs for clinical applications, it is necessary to reduce or completely eliminate the use of animal-derived materials. To achieve this, we selected the StemFit™ medium, which does not contain animal-derived materials. This culture system is a promising method for manufacturing clinical-grade hiPSCs. In addition, it is necessary to use defined culture system for the source of iPS cells, such as fibroblasts or blood cells[13,23].

Ff-hiPSCs were generated from skin-derived fibroblasts and blood-derived cells. Although the efficiency of Ff-hiPSC generation was lower than that of iPSCs established on feeder cells, we were able to obtain an adequate number of Ff-hiPSC clones. The cord blood cells appeared to be more potent sources for Ff-hiPSC generation. Almost all Ff-hiPSCs have differentiation potential, and can differentiate into several kinds of somatic-type cells. These findings indicate that the Ff-hiPSCs established under Ff and Xf conditions from several types of somatic cells are similar to the hiPSCs established using the conventional system with feeders.

In conclusion, the present results indicate that hiPSCs with equivalent growth and differentiation potential to feeder-grown hiPSCs can be generated from various human tissue samples under Ff and Xf conditions. Since this method is easy to use, expandable and reproducible, it should prove valuable for generating clinical-grade hiPSCs intended for drug screening and therapeutics, or even basic research applications.

Methods

Production of laminin-511 E8. Recombinant LN511E8 were expressed in 293-F cells (Life Technologies) with 6 × His, HA and FLAG tags at the N-termini of the α5E8, β1E8 and γ1E8 chains, respectively, and were purified using affinity chromatography with Ni-NTA resin and anti-FLAG antibody agarose, as described previously[24]. rLN511E8 was also produced in cGMP-banked Freedom CHO-S cells (Life Technologies) with an N-terminal 6 × His tag on the α5E8 chain (iMatrix-511™, Nippi, Inc.). The cGMP-compatible rLN511E8 was purified using Ni-NTA affinity chromatography and ion-exchange chromatography. cGMP-compatible rLN511E8 was manufactured in a controlled clean room (Nippi and Yokohama Biopharmaceutical Research and Development Center).

Components of StemFit™. In this study, we used newly developed medium manufactured by Ajinomoto Co., Inc., Japan. StemFit™ contains twenty-one amino acids (L-Alanine, L-Arginine, L-Asparagine, L-Aspartic Acid, L-Cysteine, L-Cystine, L-Glutamic Acid, L-Glutamine, Glycine, L-Histidine, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Phenylalanine, L-Proline, L-Serine, L-Threonine, L-Tryptophan, L-Tyrosine and L-Valine), ten vitamins (L-Ascorbic Acid, Cobalamin, Biotin, Folic Acid, I-inositol, Niacinamide, D-Calcium Pantothenate, Pyridoxine Hydrochloride, Riboflavin and Thiamine Hydrochloride), five trace minerals (Cupric Sulfate, Ferric Sulfate, Ferric Nitrate, Zinc Sulfate and Sodium selenite) and growth factors, including bFGF. All ingredients are highly defined or purified materials, and none are derived from non-human animal sources. Large-scale manufacturing of StemFit™ has been achieved in well-validated and -qualified factories in Japan.

Feeder-free culture system for hESCs and hiPSCs. The hESCs/iPSCs were cultivated on rLN511E8-coated (0.5 μg/cm²) cell culture plates with StemFit™ and passaged via dissociation into single cells using TrypLE Select (Life Technologies). Although this approach is similar to a previously published method[12], we separately developed a single cell culture system for hESCs (H9[3] and KhES1[25]) and hiPSCs (201B7[2] and 32R1[18]). Before plating the cells, cell culture dishes or plates were coated with rLN511E8 (0.5 μg/cm²). The rLN511E8 solution was diluted with PBS(−) and the diluents were transferred to cell culture dishes or plates. The dishes or plates were incubated for one hour in a CO_2 incubator (37°C). The hESCs/iPSCs were dissociated into single cells by treatment with 0.5× TrypLE Select (1× TrypLE Select diluted 1:1 with 0.5 mM EDTA/PBS(−)) for four minutes at 37°C. Since the cells remained attached to the rLN511E8-coated plates, we aspirated the 0.5× TrypLE Select and carefully washed the cells with PBS(−). The cells were scraped under the StemFit™ and dissociated into single cells by pipetting the cells 10 times. The number of dissociated single cells was counted using a Countess Automated Cell Counter (Life Technologies). We typically plated 1.3 × 10⁴ live cells into the rLN511E8-coated wells of a 6-well cell culture plate (=1.35 × 10³ live cells/cm²). A Rock inhibitor (Y-27632, final concentration 10 μM) was used only at the time of plating[26]. The next day, the medium was changed to fresh StemFit™ without Y-27632. The medium was changed every other day. Eight to ten days after plating, the cells reached 80–90% confluency and were ready for the next passage. Frozen stocks of Ff-hiPSC were similarly prepared as single cells and frozen in STEM-CELLBANKER (1 × 10⁶ live cells/mL) (Nippon Zenyaku Kogyo) at −80°C using a standard slow-freezing method.

Establishment of Ff-hiPSCs from fibroblasts and blood cells. Human primary fibroblasts were derived from biopsied skin tissue samples. The fibroblasts were established and expanded with DMEM containing 10% autologous human serum. Using these fibroblasts, iPS cells were generated as described previously[17]. Briefly, following electroporation of reprogramming factors with episomal vectors using the Neon system (Life Technologies), the cells were plated on a non-coated cell culture plate. iPS cells were induced by changing the medium to StemFit™. Twenty to thirty days after plating, iPS cell colonies were observed.

Blood cell-derived iPS cells were generated as described previously[16]. Briefly, mononuclear cells were prepared from peripheral blood using the Ficoll-Paque PREMIUM (GE Healthcare) separation method. The cells were electroporated with episomal vectors using a Nucleofector 4D system (with P3 Primary Cell Kit, Lonza) and plated on rLN511E8-coated cell culture plates. The iPSCs were induced by changing the medium to StemFit™. Twenty to thirty days after plating, iPS cell colonies were observed. A similar method was used to generate Ff-hiPSCs from human cord blood (provided by the RIKEN Bioresource Center Cell Bank). We generated several clones of Ff-hiPSCs from each experiment.

The experimental protocols dealing with human subjects were approved by the institutional review board at our institute (Kyoto University Graduate School and Faculty of Medicine, Ethics Committee). Written informed consent was provided by each donor.

Genomic PCR analysis of the integration of the episomal vectors. Purified genomic DNA was used as a template to detect the remaining episomal vectors in the hiPSCs using a PCR analysis. The episomal vectors were detected using the primer set for EP4. The EP4 primer set was constructed in the EBNA1 region. The Fbx15 gene was amplified with a primer set for Fbx as an internal control. The episomal vector, pCXLE-EGFP, was used as a positive control ("Plasmid"). The primer sets are listed in Supplementary Table 3.

Antibodies used for immunostaining. The antibodies used in this study included anti-Oct3/4 (BD, 611202), -Nanog (R&D, AF1997), -SSEA4 (BD, 560073), -TRA-1-60 (BD, 560071), -Sox17 (R&D, AF1924), -α-smooth muscle actin (SMA) (Dako, M0851), -α-1-fetoprotein (AFP) (Dako, N1501) and -βIII tubulin (Millipore, MAB1637) antibodies.

RNA isolation and reverse transcription. Total RNA purification and RT-PCR were performed as described previously[2,27–29]. The expressions of Nanog, Sox2, Oct3/4, Lin28, L-Myc, c-Myc, Klf4 and G3PDH were detected using the primer sets listed in Supplementary Table 3.

***In vitro* differentiation.** To examine whether Ff-hiPSCs spontaneously differentiate into the three germ layers, the Ff-hiPSCs were cultivated with StemFit™ lacking bFGF (StemFit™-bFGF) for two weeks on rLN511E8-coated cell culture plates. The differentiated cells were immunostained with the indicated antibodies.

Directed differentiation into dopaminergic neurons. Dopaminergic neurons were induced from Ff-hiPSCs under xeno-free conditions. The dissociated Ff-hiPSCs were plated on rLN511E8-coated plates at high density (7.5 × 10⁵ cells/cm²; Day 0). Glasgow minimum essential medium (GMEM, Life Technologies) was supplemented with 8% Xeno-free knockout serum replacement (Life Technologies), sodium pyruvate (SIGMA), 2-mercapto ethanol (Wako), MEM NEAA (Life Technologies), 500 nM of A-83-01 (Activin inhibitor, Wako), 100 nM of LDN193189 (BMP inhibitor, STEMGENT) and 10 μM Y-27632 (Wako). To induce neural cells with a ventral mesencephalic phenotype, 2 μM Purmorphamine (Wako) and 100 ng/mL of FGF-8 (Wako) were added starting the next day. Starting on day 3, 3 μM CHIR99021 (GSK3β inhibitor, Wako) was also added. On day 12, the first passage was performed with TrypLE CTS (Life Technologies), and the neural cells were replated on a fresh rLN511E8 surface at the same density as on day 0. The basal medium was switched to Neurobasal medium supplemented with Xeno-free B27 (Life Technologies), 200 μM of ascorbic acid (SIGMA), 400 μM of dbc AMP (SIGMA), 2 ng/mL of GDNF (R&D), 20 ng/mL of BDNF (R&D) and 10 μM Y-27632. Throughout the neural induction process, the medium was changed every day. On days 12, 20 and 28, the culture was passaged onto a new rLN511E8 surface. Y-27632 was added to the medium on the day of passage. Immunostaining was performed using anti-TH (Chemicon, AB152), -Foxa2 (Santa Cruz, SC6554) and –Nurr1 (kindly provided by KAN Research Institute) antibodies.

Directed differentiation into blood cells. Ff-hiPSCs were differentiated into blood cells as described previously[30] with some key modifications. Briefly, Ff-hiPSCs were cultivated in Ultra Low Attachment 6-well cell culture plates (Corning) with StemFit™ to prepare EB-like spheres. The spheres were subsequently used for blood cell differentiation according to the established protocol[20].

Directed differentiation into insulin-producing cells. Ff-hiPSCs were differentiated into insulin-producing cells as described previously[21]. Briefly, human Ff-hiPSCs were dissociated and plated on rLN511E8 and cultured with the reported factors required for pancreatic differentiation. The dissociated cells were treated with 100 ng/ml of activin A and 3 μM CHIR99021 for 24 hours, and then were treated with 100 ng/ml of activin A and 1 μM CHIR99021 for four days. Next, the cells were cultured with 1 μM dorsomorphin (Calbiochem, San Diego, CA), 2 μM retinoic acid (Sigma, St. Louis, MO) and 10 μM SB431542 (Sigma) for six days. Subsequently, the cells were cultured with 10 μM forskolin, (Wako), 10 μM dexamethasone (Wako), 5 μM Alk5 inhibitor II (Calbiochem) and 10 mM nicotinamide (StemCell Technologies, Vancouver, BC) for 12 days. Following the culture, the cells were fixed and stained with Hoechst33342 (Life Technologies) and antibodies against insulin (Dako).

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Author contributions

Conceived and designed the experiments: M. Nakagawa, S.S. and S.Y. Performed the experiments: M. Nakagawa, S.S., Y.T., N.T., T.I., K.A., A.M., J.T., T.T., K.O., M. Nishizawa, Y.Y. and K.S. Analyzed the data: M. Nakagawa, S.S. and Y.T. Contributed reagents/materials/analysis tools: M. Nakagawa, S.S., Y.T. and K.S. Wrote the paper: M. Nakagawa.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

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Making Steady Progress on Direct Cardiac Reprogramming Toward Clinical Application

Kenji Miki, Yoshinori Yoshida and Shinya Yamanaka

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Making Steady Progress on Direct Cardiac Reprogramming Toward Clinical Application

Kenji Miki, Yoshinori Yoshida, Shinya Yamanaka

Reprogramming of Human Fibroblasts Toward a Cardiac Fate
Nam et al
Proc Natl Acad Sci USA. 2013;110:5588–5593.

A new report demonstrates direct cardiac reprogramming in human cells for the first time and points to the possibility of moving this technology toward clinical applications.

Direct reprogramming to somatic cells by forced expression of a combination of lineage-specific transcription factors or micro RNAs (miRNAs) has been demonstrated in a variety of rodent cell types, such as neurons,[1] neural stem cells,[2] hepatocyte-like cells,[3,4] β-cells,[5] and cardiomyocytes.[6–10] Three groups have reported the direct neuronal reprogramming of human fibroblast into neuronal cells.[11–13] Pang et al[11] demonstrated that the same 3 transcription factors used for mouse cells, Brn2, Ascl1, and Myt1l, are insufficient to induce functional neurons from human fetal fibroblasts, but that Brn2, Ascl1, and Myt1l plus the addition of NenroD1 can convert human fetal and postnatal fibroblasts into functional neurons. Yoo et al[13] also reported that a combination of neuronal transcription factors and 2 miRNAs, micro RNA (miR)-9/9* and miR-124, can efficiently convert human fibroblasts into functional neurons. These findings suggest that, compared with mouse cells, additional factors might be required to direct the reprogramming of human somatic cells into other lineage cells.

More recently, Nam et al[14] reported the direct reprogramming of human fibroblasts into cardiac-like cells (iCLMs). They first tested whether the same cardiac transcription factors, Gata4, Mef2c, and Tbx5 (GMT) or GMT+Hand2, which were previously reported to be useful for the direct reprogramming of mouse fibroblasts into cardiomyocytes,[6–8,10] could reprogram neonatal human foreskin fibroblasts into iCLMs. However, GMT and GMT+Hand2 both failed to induce cardiac marker expression in human foreskin fibroblasts. To determine the optimal combination of factors for the direct cardiac reprogramming of human fibroblasts, they examined the effects of additional factors, including other cardiac transcription factors and miRNAs, with GMT+Hand2. They found that a combination of Gata4, Hand2, Tbx5, Myocardin, miR-1, and miR-133 (6F) could convert human fibroblasts into iCLMs. The transduction of 6F induced ≈20% of human foreskin fibroblasts, ≈13% of adult human cardiac fibroblasts, and 9.5% of adult human dermal fibroblasts to express cardiac Troponin T 2 weeks later. Furthermore, spontaneous contractions were observed in a small subset of iCLMs derived from adult human cardiac fibroblasts 11 weeks later, but not from either human foreskin fibroblasts or adult human dermal fibroblasts.

In this study, they identified a combination of factors, 6F, that are capable of direct cardiac reprogramming from human fibroblasts. Interestingly, the transduction of Mef2c, which is one of the key factors used to reprogram mouse fibroblasts directly into cardiomyocytes,[6–8,10] with 6F significantly decreased the percentage of cardiac Troponin T$^+$ cells. They previously showed that miR-1-1/133a-2 and miR-1-2/133a-1 were downregulated in the hearts of mice lacking MEF2 expression and a MEF2-dependent upstream enhancer of the miR-1-1/133a-2 has been shown to regulate miR-1-1/133a-2 expression in cardiac and skeletal muscle in vivo.[16,17] Therefore, they mentioned that Mef2c regulates the expression of miR-1 and miR-133,[15] and that these miRNAs probably play an alternative role of Mef2c.

The precise molecular mechanism(s) underlying the direct cardiac reprogramming from fibroblasts is still unknown even in mice, and there is a possibility that there might be a mechanistic difference in the reprogramming process between mouse and human cells.

Regarding the direct reprogramming to somatic cells, the reprogramming efficiency and reproducibility are very important and often controversial subjects. A previous study by Chen et al[18] showed that forced expression of GMT in mouse tail tip fibroblasts and cardiac fibroblasts was insufficient to induce cardiac phenotypes. Nam et al[14] also showed that forced expression of a combination of miR-1, miR-133, miR-208 and miR-499, which were previously reported to be useful for the direct cardiac reprogramming of mouse fibroblasts,[9] failed to induce the expression of cardiac markers in mouse tail-tip fibroblasts. These findings suggest that an accumulation of slight differences among laboratories, such as the culture and isolation methods used for the fibroblasts, the fibroblast lines used or the method of virus production, might cause differences in the reprogramming efficiency and reproducibility. As indicated above, Nam et al[14] first reported the direct cardiac reprogramming of human fibroblasts. In their study, cardiac Troponin T$^+$ cells at 2 weeks and calcium transients at 4 weeks after the transduction of 6F were observed in ≈13% and ≈15% of adult human cardiac fibroblasts, respectively. However, spontaneous contractions were observed in only a few iCLMs derived from adult human cardiac fibroblasts. Many other researchers are

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From the Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan (K.M., Y.Y.); and Center for iPS Cell Research and Application and Gladstone Institute of Cardiovascular Disease, San Francisco, CA (S.Y.).

Correspondence to Yoshinori Yoshida, MD, PhD, Center for iPS Cell Research and Application, Kyoto University, 53 Shogoin-Kawaharacho, Sakyo-ku, Kyoto, 606–8507, Japan. E-mail yoshinor@cira.kyoto-u.ac.jp

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also attempting to reprogram human somatic cells directly into cardiomyocytes, and thus, further improvements to achieve a high reprogramming efficiency are expected, and it is likely that the reproducibility of the method of Nam et al[14] will be demonstrated by another group in the near future.

The reprogrammed iCLMs from human fibroblasts were functionally immature. In the case of mouse cells, Inagawa et al[10] reported that the in vivo transduction of a polycistronic retrovirus vector expressing GMT separated by 2A peptides (3F2A) generated more mature cardiomyocytes compared with the in vivo transduction of GMT. In addition, some studies suggested that the in vivo environment, such as the presence of mechanical force and extracellular matrix and secreted proteins, might be more suitable for direct reprogramming with regard to the reprogramming efficiency, reprogrammed cell survival, and maturation.[5,7,8] The further development of gene delivery systems, such as polycistronic vectors, and the use of the in vivo environment might help to reprogram human fibroblasts into more mature cardiomyocytes.

In addition, the reprogrammed iCLMs from human fibroblasts were heterogeneous. In fact, the expression levels of cardiac and noncardiac genes in the iCLMs varied widely and only a small subset of iCLMs showed spontaneous contraction. It is supposed that partially reprogrammed cells exist in the population of reprogrammed cells, and such partially reprogrammed cells might cause arrhythmias in the heart. The authors mentioned that this heterogeneity of iCLMs is because of variations in the stoichiometry and levels of the expression of factors in individual cells and because of the heterogeneity of the original fibroblasts. In the future, improvements in the reprogramming efficiency and gene introduction methods may reduce the heterogeneity and thereby make the clinical application of this technology more feasible.

Attractive approaches, including direct cardiac reprogramming, have been developed in recent years with the goal of future cardiac regeneration therapy. Human pluripotent stem cells (PSCs), such as embryonic stem cells and induced PSCs, which can efficiently differentiate into cardiomyocytes after the addition of a combination of growth factors and are able to provide large amount of cardiomyocytes, are promising candidate cell sources. The transplantation of cardiomyocytes derived from human PSCs has already been reported to improve the cardiac function in rat and in swine infarction models.[19,20] In addition, Shiba et al[21] reported that human embryonic stem cell–derived cardiomyocytes can electrically couple and suppress arrhythmias in injured guinea pig hearts. However, many problems still remain to be resolved before the clinical application of human PSCs for cardiac disease. In particular, the elimination of undifferentiated PSCs to avoid tumor formation and the preparation of a large amount of cardiomyocytes derived from human PSCs are very important issues. The direct cardiac conversion from somatic cells is also a promising approach for cardiac regeneration therapy and may overcome the risk of tumor formation. Some groups have already reported the in vivo direct cardiac reprogramming of cardiac fibroblasts in the mouse heart.[7–10] In addition, Qian et al[7] and Song et al[8] revealed that the transduction of cardiac transcription factors in vivo improved the cardiac function and reduced fibrosis after myocardial infarction. Before the clinical application of this technology, the full effects of direct cardiac conversion and the safety of the transduction methods using defined factors must be demonstrated in large animal models. In addition, quantitative comparison of cardiomyocytes generated by direct cardiac conversion technology and PSC-derived cardiomyocytes will be required. Although direct cardiac conversion is still a developing technology, this new technology possesses great potential for future cardiac regeneration therapy.

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Disclosures

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ORIGINAL ARTICLE
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Ultrastructural Maturation of Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes in a Long-Term Culture

Tsukasa Kamakura, MD; Takeru Makiyama, MD, PhD; Kenichi Sasaki, MD; Yoshinori Yoshida, MD, PhD; Yimin Wuriyanghai; Jiarong Chen; Tetsuhisa Hattori, MD, PhD; Seiko Ohno, MD, PhD; Toru Kita, MD, PhD; Minoru Horie, MD, PhD; Shinya Yamanaka, MD, PhD; Takeshi Kimura, MD, PhD

Background: In the short- to mid-term, cardiomyocytes generated from human-induced pluripotent stem cells (hiPSC-CMs) have been reported to be less mature than those of adult hearts. However, the maturation process in a long-term culture remains unknown.

Methods and Results: A hiPSC clone generated from a healthy control was differentiated into CMs through embryoid body (EB) formation. The ultrastructural characteristics and gene expressions of spontaneously contracting EBs were analyzed through 1-year of culture after cardiac differentiation was initiated. The 14-day-old EBs contained a low number of myofibrils, which lacked alignment, and immature high-density Z-bands lacking A-, H-, I-, and M-bands. Through the long-term culture up to 180 days, the myofibrils became more tightly packed and formed parallel arrays accompanied by the appearance of mature Z-, A-, H-, and I-bands, but not M-bands. Notably, M-bands were finally detected in 360-day-old EBs. The expression levels of the M-band-specific genes in hiPSC-CMs remained lower in comparison with those in the adult heart. Immunocytochemistry indicated increasing number of MLC2v-positive/MLC2a-negative cells with decreasing number of MLC2v/MLC2a double-positive cells, indicating maturing of ventricular-type CMs.

Conclusions: The structural maturation process of hiPSC-CMs through 1-year of culture revealed ultrastructural sarcomeric changes accompanied by delayed formation of M-bands. Our study provides new insight into the maturation process of hiPSC-CMs. (*Circ J* 2013; **77:** 1307–1314)

Key Words: Cardiomyocytes; Induced pluripotent stem cells; Ultrastructure

Induced pluripotent stem cells (iPSC) can differentiate into functional cardiomyocytes (CMs), and are a powerful model for regenerative therapy and investigating the mechanisms underlying inherited cardiac diseases.[1–5] Although several studies have shown that iPSC-derived CMs (iPSC-CMs) have molecular, structural and functional properties resembling those of adult CMs,[6–9] they have proved to be less mature than adult and fetal CMs.[10–12] Thus, there is limited information about the electrophysiological and biochemical properties of iPSC-CMs, and the ultrastructural maturation process has not been investigated fully.

The ultrastructural features of human iPSC-CMs (hiPSC-CMs) at around 30 days after cardiac differentiation have been described as being similar to those of adult CMs showing myofibrillar bundles with transverse Z-bands.[4,13,14] However, in those reports, hiPSC-CMs still remain embryonic in phenotype, lacking a mature sarcomeric structure with M-bands and a variable degree of myofibrillar organization. It is unknown whether hiPSC-CMs can develop the adult CM-like ultrastructure in vitro. Immaturity of the hiPSC-CMs may hamper their application for studying cardiac diseases, drug development, and regenerative medicine, and could affect functional properties and drug responses in vitro and increase the risk of abnormal growth in vivo. Therefore, it is crucial to elucidate the

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Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto (T. Kamakura, T.M., K.S., Y.W., J.C., T. Kimura); Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Otsu (T.H., S.O., M.H.); Center for iPS Cell Research and Application (CiRA), Institute for Integrated Cell-Material Sciences Kyoto University, Kyoto (Y.Y., S.Y.); and Department of Cardiovascular Medicine, Kobe City General Hospital, Kobe (T. Kita), Japan

Mailing addresses: Takeru Makiyama, MD, PhD, Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. E-mail: makiyama@kuhp.kyoto-u.ac.jp and Yoshinori Yoshida, MD, PhD, Center for iPS Cell Research and Application (CiRA), Institute for Integrated Cell-Material Sciences, Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. E-mail: yoshinor@cira.kyoto-u.ac.jp

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