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An iPSC line derived from a human acute myeloid leukemia cell line (HL-60-iPSC) retains leukemic abnormalities and displays myeloid differentiation defects

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Abstract

Cancer-derived iPSCs have provided valuable insight into oncogenesis, but human cancer cells can often be difficult to reprogram, especially in cases of complex genetic abnormalities. Here we report, to our knowledge, the first successful generation of an iPSC line from a human immortalized acute myeloid leukemia (AML) cell line, the cell line HL-60. This iPSC line retains a majority of the leukemic genotype and displays defects in myeloid differentiation, thus providing a tool for modeling and studying AML.

1. Resource utility

Reprogramming has enabled further understanding of the development and progression of cancer, including leukemogenesis. Here we report an iPSC line generated from the immortalized AML cancer cell line HL-60. HL-60-iPSCs retain leukemic abnormalities of the parental line, and can serve as a valuable model to study AML.

2. Resource details

Cells from the HL-60 leukemic cell line (Gallagher et al., 1979; Dalton et al., 1988) were reprogrammed by infection with Sendai virus expressing the four Yamanaka factors *(OCT4/POU5F1, SOX2, KLF4, c*-MYC), and colonies were manually picked based on iPSC-like morphology approximately four weeks after infection. Only one iPSC line was successfully

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2020.102096.

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expanded in culture (Fig. 1A) and was evaluated for pluripotency based on gene and cell surface marker expression, as well as protein expression, compared to a positive control iPSC line (Fig. 1B-D). Clearance of the Sendai virus vector was also confirmed (Fig. 1E).

Given the low reprogramming efficiency and the genomic complexity of the parental line, we examined genomic integrity and confirmed iPSC identity with the genome-wide HumanCore BeadChip SNP array (D'Antonio et al., 2017; Panopoulos et al., 2017) (Fig. 1F). [Short Tandem Repeat (STR) analysis also confirmed the iPSC line was derived from HL-60 cells; submitted in archive with journal]. The SNP array demonstrated that the HL-60-iPSC line retained almost all of the genomic aberrations of the parental HL-60 leukemic line, despite the wide array of abnormalities present (Fig. 1F; HL-60-iPSCs were analyzed at passage 12; genomic abnormalities present in both parental line and iPSC line are shaded in grey; Note: it is possible that the few parental HL-60 cell line mutations not detected in iPSCs are still present in low levels in the HL-60-iPSCs, but that they are now outside the detection limits of the array) (D'Antonio et al., 2017).

Despite the variety of genomic aberrations, HL-60-iPSCs demonstrated functional pluripotency *in vitro* by successful differentiation into embryonic germ layers. Embryoid body-mediated differentiation followed by immunofluorescent analysis indicated positive staining for all germ layers (Fig. 1G). Additional analysis of *in vitro* differentiation potential was performed by real-time PCR of germ-layer specific genes (D'Antonio et al., 2017), which also demonstrated that the HL-60-iPSC line has the ability to generate cells expressing genes for all three embryonic germ layers at levels comparable to control iPSCs (Fig. 1H). Given their leukemic origin, to determine if HL-60-iPSCs demonstrated a specific differentiation deficiency in their ability to produce mature myeloid cells, HL-60-iPSCs were subjected to directed differentiation towards mature neutrophils. When compared to a control iPSC line for expression of the cell surface marker CD16, HL-60-iPSCs show a clear defect in the generation of CD16-expressing myeloid cells (Fig. 1I; Table 1).

Our results demonstrate, to our knowledge, the first successful generation of iPSCs from a human immortalized AML cell line. These findings also provide evidence that it is possible to generate iPSCs with widespread genomic aberrancies (albeit at low reprogramming efficiencies). Thus, HL-60-iPSCs provide a unique tool for modeling and studying this hematologic malignancy.

3. Materials and methods

3.1. Cell lines

HL-60 peripheral blood leukemia cells were obtained from ATCC (ATCC® CCL240™) (Gallagher et al., 1979; Dalton et al., 1988). Control iPSC lines have been previously published (Panopoulos et al., 2017).

3.2. Generation of iPSCs

Minor modifications were made to the manufacturer's suggested protocol using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit: 2.0×10^5 HL-60 leukemic cells were

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infected at twice the recommended MOI. Resultant iPSCs were expanded and maintained in mTeSR™1 on Matrigel®.

3.3. Flow cytometry

A BD LSRFortessa™ X-20 was used to analyse cells labelled with fluorescently-conjugated antibodies, and figures generated in FlowJo (Table 2).

3.4. Gene expression analysis and gel electrophoresis

Total RNA was isolated using Trizol and cDNA for RT-qPCR generated with the iScript™ Reverse Transcription Supermix. PCR analysis of Sendai virus clearance was performed using the Phusion® High-Fidelity PCR Master Mix, using 35 cycles of 95 °C for 10 s, 64 °C for 30 s, 72 °C for 10 s. Products were analysed on a 2% agarose gel with a 100 bp DNA standard, using previously-infected cells as a positive control for presence of Sendai virus (Table 2).

3.5. Karyotype and identify analysis

Genomic analysis and identity testing was performed using the HumanCore Exome array, which has a resolution of ~ 100 kb, as previously described (D'Antonio et al., 2017). DNA was isolated using the DNeasy Blood and Tissue kit, and 200 ng of genomic DNA hybridized to HumanCore-24 BeadChips. Chips were processed according to manufacturer's suggested protocol on a HiScan, with genotype calls, log R ratios (LRR), and B-allele frequencies (BAF) extracted using GenomeStudio V2011.1. Distribution of LRR and BAF were used to manually observe copy number aberrant regions, and the estimated boundary position for each aberration determined using specific probe locations. Abbreviations in Fig. 1F: chr = chromosome; DEL = deletion; DUP = duplication; LOH = loss of heterozygosity. PCR amplification of D17S1290 loci was performed as described above and used for STR analysis.

4. Undirected *in vitro* differentiation

To generate uniform embryoid bodies (EBs), small dots of Matrigel® were plated onto 10-cm cell culture dishes. HL-60-iPSCs were individualized using TrypLE and plated onto Matrigel® dots in mTeSR™1, with subsequent media changes every day until dots were solid iPSC colonies. Colonies were then detached from the 10-cm plate using dispase and plated into T25 low-attachment flasks in EB media (DMEM/F12, 10% FBS, 0.5 mM L-glutamine, 0.1 mM non-essential amino acids, 55 mM P-mercaptoethanol), where they formed compact EBs. After 4 days of suspension culture with daily media changes, EBs were plated onto gelatin-coated 24-well plates and cultured for an additional 10 days in EB media. Differentiated cells were either collected from wells for RNA isolation using TrypLE or fixed and stained for microscopy.

5. Myeloid differentiation

HL-60-iPSCs were subjected to directed myeloid differentiation by co-culture with mouse OP9 stromal cells in media containing cytokines (Choi et al., 2011). In brief, iPSCs were

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differentiated into multipotent myeloid progenitors via coculture over 9 days, with half-media changes on days 4 and 6. Myeloid progenitors were then expanded in low-attachment T25 flasks for 2 days in the presence of GM-CSF, and further differentiated into neutrophils in the presence of G-CSF. Analysis was performed via flow cytometry as described above.

6. Mycoplasma testing

Absence of mycoplasma in the HL-60-iPSC line was confirmed using the Lonza MycoAlert™ detection kit using manufacturer's protocol.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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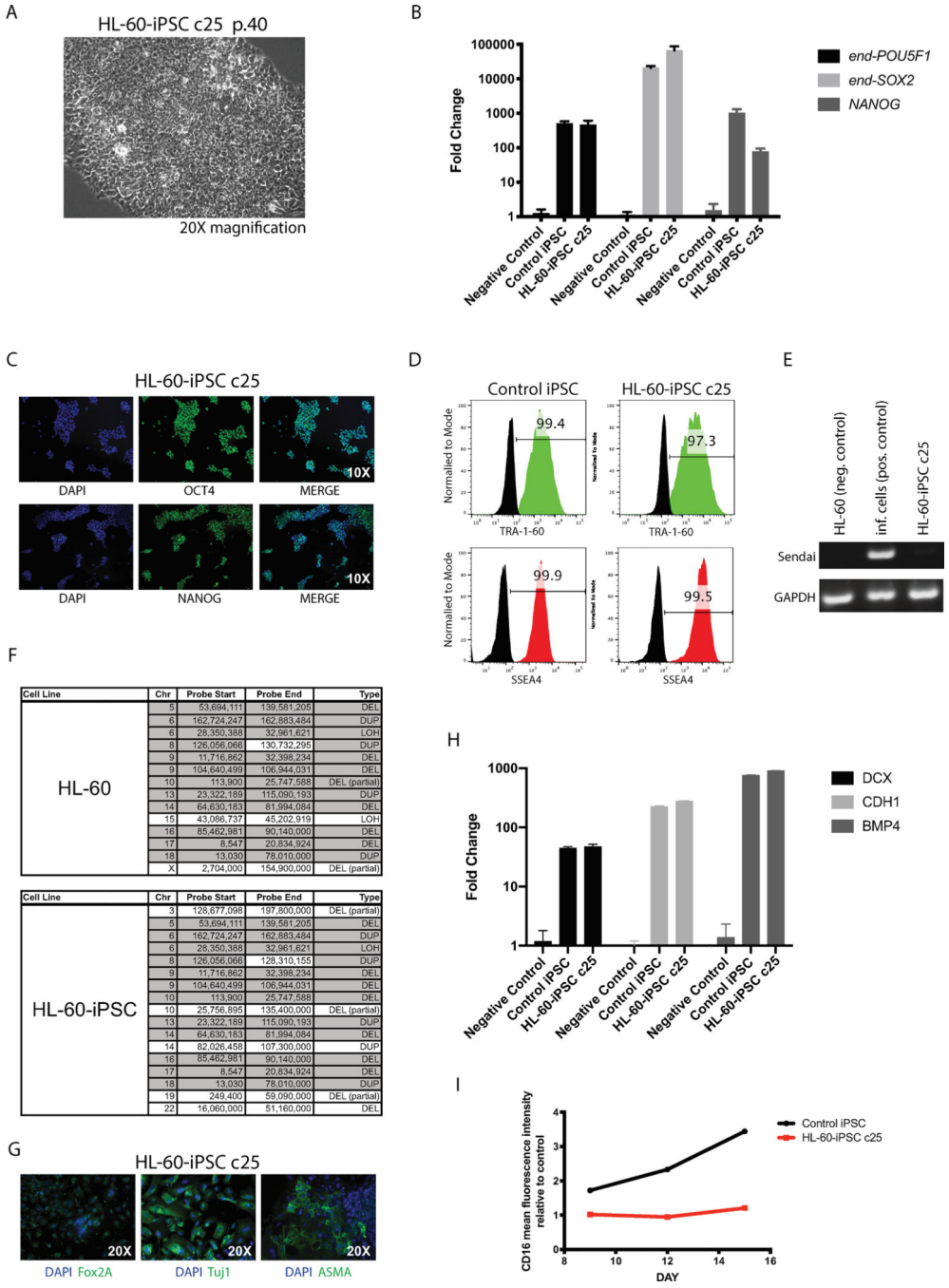

Cell Line	Chr	Probe Start	Probe End	Type
HL-60	5	53,694,111	139,581,205	DEL
	6	162,724,247	162,883,484	DUP
	6	28,350,388	32,961,621	LOH
	8	126,056,066	130,732,295	DUP
	9	11,716,862	32,398,234	DEL
	9	104,640,499	106,944,031	DEL
	10	113,900	25,747,588	DEL (partial)
	13	23,322,189	115,090,193	DUP
	14	64,630,183	81,994,084	DEL
	15	43,086,737	45,202,919	LOH
	16	85,462,981	90,140,000	DEL
	17	8,547	20,834,924	DEL
	18	13,030	78,010,000	DUP
	X	2,704,000	154,900,000	DEL (partial)

Cell Line	Chr	Probe Start	Probe End	Type
HL-60-iPSC	3	126,677,098	197,800,000	DEL (partial)
	5	53,694,111	139,581,205	DEL
	6	162,724,247	162,883,484	DUP
	6	28,350,388	32,961,621	LOH
	8	126,056,066	128,310,155	DUP
	9	11,716,862	32,398,234	DEL
	9	104,640,499	106,944,031	DEL
	10	113,900	25,747,588	DEL
	10	25,756,895	135,400,000	DEL (partial)
	13	23,322,189	115,090,193	DUP
	14	64,630,183	81,994,084	DEL
	14	82,026,458	107,300,000	DUP
	16	85,462,981	90,140,000	DEL
	17	8,547	20,834,924	DEL
	18	13,030	78,010,000	DUP
	19	249,400	59,090,000	DEL (partial)
	22	16,060,000	51,160,000	DEL

Fig. 1.
Generation and characterization of HL-60-iPSCs.

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Table 1

Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Large nuclear:cytoplasmic ratios and colony shapes characteristic of normal iPSCs	Fig. 1, panel A
Phenotype	Qualitative analysis (immunofluorescence)	Positive staining for Oct4, Nanog	Fig. 1, panel C
	Quantitative analysis (flow cytometry, qPCR)	Surface marker expression: Tra-1–60: 97.3%, SSEA4: 99.5%; Gene expression: Oct4, Nanog, Sox2: >50-fold upregulation	Fig. 1, panels B, D
Genotype	Karyotype and resolution	HumanCore Exome array resolution ~ 100 kb	Fig. 1, panel F
Identity	Microsatellite PCR (mPCR) OR STR analysis	DNA profiling not performed	N/A
		STR analysis of D17S1290 locus matched; HumanCore Exome array also performed	Submitted in archive with journal; Fig. 1, panel F
Mutation analysis (IF APPLICABLE)	Sequencing	Complex genotype	Fig. 1, panel F
	Southern Blot OR WGS	Not performed	N/A
Microbiology and virology	Mycoplasma	Negative by luminescence testing	Supplementary Fig. 1
Differentiation potential	Embryoid body formation (immunofluorescence and qPCR)	Immunofluorescence: forkhead box A2, β-tubulin III, a smooth muscle actin; qPCR: doublecortin (ectoderm); cadherin-1 (endoderm); bone morphogenetic protein 4 (mesoderm)	Fig. 1, panels G, H
	Directed differentiation (flow cytometry)	CD16 (mature myeloid cells)	Fig. 1, panel I
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	HL-60 parental line: Negative	Not shown, but available with author
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

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Table 2

Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency markers (flow)	Mouse anti-TRA-1-60 FITC	1:20	BD Pharmingen Cat# 560173, RRID: AB_1645379
Pluripotency markers (flow)	Mouse anti-SSEA-4 PE	1:20	BD Pharmingen Cat#560128, RRID: AB_1645533
Pluripotency markers (IF)	Rabbit anti-Oct4	1:200	Abcam Cat#ab19857, RRID: AB_445175
Pluripotency markers (IF)	Rabbit anti-Nanog	1:100	Abcam Cat# ab21624, RRID: AB_446437
Myeloid marker (flow)	Mouse anti-CD16 PE	1:20	Life Technologies Cat#MHC1604, RRID: AB_10373687
Differentiation markers (IF)	Mouse anti-alpha smooth muscle actin	1:100	Abcam Cat# ab7817, RRID:AB_262054
Differentiation markers (IF)	Mouse anti-forkhead box A2	1:100	Abnova Cat# H00003170-M12, RRID: AB_626426
Differentiation markers (IF)	Mouse anti-beta III tubulin	1:100	BioLegend Cat# 657402, RRID: AB_2562570
Secondary antibodies (IF)	CF488A chicken anti-mouse IgG (H + L)	1:500	Biotium Cat# 20208, RRID: AB_10853798
Primers			
	Target	**Forward/Reverse primer (5'–3')**	
Pluripotency markers (qPCR)	Endogenous POU5F1	GGGTTTTTGGGATTAAGTTCTTCA/ GCCCCCACCCTTTGTGTT	
Pluripotency markers (qPCR)	Endogenous SOX2	CAAAAATGGCCATGCAGGTT/ AGTTGGGATCGAACAAAAGCTATT	
Pluripotency markers (qPCR)	NANOG	ACAACTGGCCGAAGAATAGCA/ GGTTCCCAGTCGGGTTCAC	
Ectoderm markers (qPCR)	DCX	TGCCTCAGGGAGTGCGTTA/ GAACAGACATAGCTTTCCCCTTC	
Endoderm markers (qPCR)	CDH1	CGAGAGCTACACGTTCACGG/ GGGTGTCGAGGGAAAAATAGG	
Mesoderm markers (qPCR)	BMP4	GCACTGGTCTTGAGTATCCTG/ TGCTGAGGTTAAAGAGGAAACG	
Housekeeping gene (qPCR)	18S	GGCGCCCCCTCGATGCTCT/ GCTCGGGCCTGCTTTGAACACTCT	
STR Analysis (PCR)	D17S1290	GCCAACAGAGCAAGACTGTC/ GGAAACAGTTAAATGGCCAA	
Reprogramming factor clearance (PCR)	Sendai virus	ATGCGGCTGATCTTCTCACT/CTTTGCCACGCAATTAGGGT	

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Resource Table:

Unique stem cell line identifier	**NDi001-A**
Alternative name(s) of stem cell line	HL-60-iPSC c25 (NDi001-A)
Institution	University of Notre Dame
Contact information of distributor	Athanasia D. Panopoulos; apanopou@nd.edu
Type of cell line	iPSCs
Origin	Human
Additional origin info	Age: 36
	Sex: Female
Cell Source	Peripheral blood-derived HL-60 cell line (ATCC®CCL240™)
Clonality	Clonal
Method of reprogramming	Sendai virus
Genetic Modification	No
Type of Modification	N/A
Associated disease	Acute myeloid leukemia
Gene/locus	Complex genotype; see Fig. 1F
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	N/A
Cell line repository/bank	Registered in the Human Pluripotent Stem Cell
	Registry (https://hpscreg.eu)
Ethical approval	Commercially purchased (ATCC)