Red blood cell generation from human induced pluripotent stem cells: perspectives for transfusion medicine

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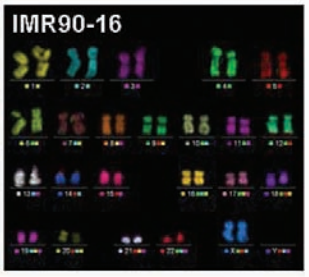
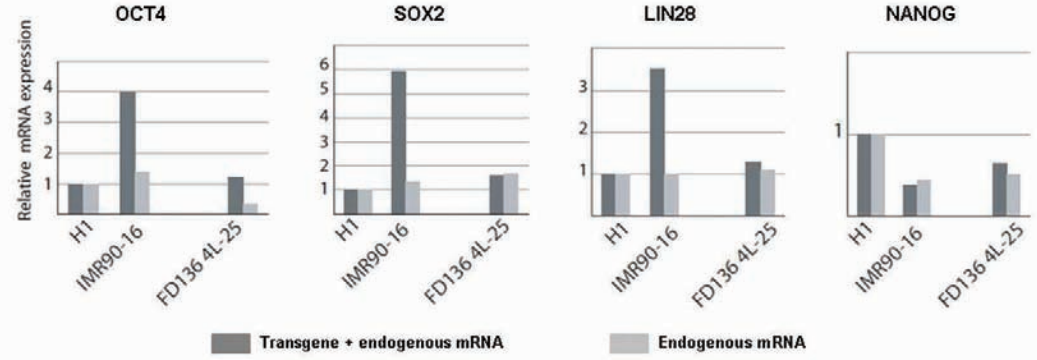
A



B

		oct-04	Oct4A	Nanog	Sox2	KLF4	DNMT	TDGF 1	GDF3	Cadh	ZFP42
	hEC 2102EP	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
IMR90	clone 16	0,49	0,12	0,01	0,46	0,01	0,18	0,09	0,34	0,36	0,13

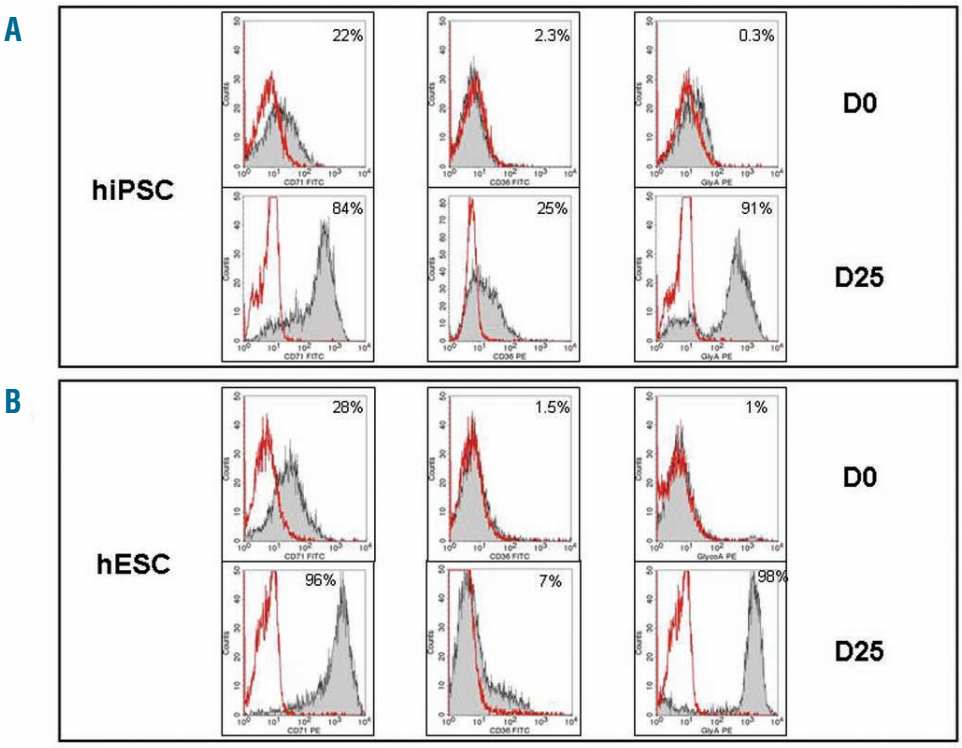
C



D

		Ectoderm		Mesoderm						Endoderm		
		Pax 6	ck18	Brachyury	Gata4	RunX 1	CD34	Nkx2.5	KDR	ck17	AFP	Gata 4
	hEC 2102EP	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
IMR90	clone 16	2,66	1,06	4,26	7,16	12,47	2,04	17,15	0,59	12,30	9,51	7,16

Online Supplementary Figure S1. hiPSC characterization. (A) hiPSC (IMR90-16, passage 10) maintain a normal karyotype. High resolution, multi-FISH karyotype indicates a normal, diploid, female chromosomal content. (B) Expression of hESC-marker genes in hiPSC (IMR90-16) clones by quantitative reverse transcription – PCR analyses (qRT-PCR). Individual PCR reactions were normalized against GAPDH and standardized relative to the expression level in human embryonic carcinomal 2102EP cells. (C) qRT-PCR analyses of OCT4, SOX2, LIN 28, and NANOG expression in fetal hiPSC lines (IMR90-16, passage 10), adult foreskin fibroblasts (FD136), adult hiPSC FD136 4L 25 passage 11 and hESC (H1). Dark gray bars represent amplification of both transgene and endogenous mRNA. Light gray bars represent the amplification of endogenous mRNA only. Data were normalized to H1 level that was set at 1. (D) Gene expression in differentiated hiPSC (IMR90-16) clones by qRT-PCR analyses. qRT-PCR performed on embryoid bodies-differentiated hiPSC shows up-regulated expression of lineage markers from all three embryonic germ layers (ectoderm: PAX6 and CK18; endoderm: GATA4, AFP and CK17; mesoderm: brachyury, GATA4, RUNX1, CD34, NKX2.5 and KDR).



Online Supplementary Figure S2. Phenotypic analyses during erythroid differentiation from hiPSC-IMR90-16 (A) and hESC-H1 (B) by flow cytometry. Expression of erythroid antigens: CD36, CD71 and Glyco-A, from day 0 after hEB dissociation to day 25 – the final point of the erythroid differentiation.

Online Supplementary Table S1. Primer sets for PCR reactions. "Endogenous" indicates that primers included in the 3′ untranslated region measure expression of the endogenous gene only, whereas "total" indicates that primers in coding regions measure expression of both the endogenous gene and the transgene if present.

Genes		Sequences (5' to 3')
OCT4 - Total	F	CTGGAGCAAAACCCGGAGGAG
	R	GCAGATGGTCGTTTGGCTGAATACC
OCT4 - Endogenous	F	AGTTTGTGCCAGGGTTTTTG
	R	ACTTCACCTTCCCTCCAACC
SOX2 - Total	F	TACCTCTTCCTCCCACTCCA
	R	GGTAGTGCTGGGACATGTGA
SOX2 - Endogenous	F	AGTCTCCAAGCGACGAAAAA
	R	TTTCACGTTTGCAACTGTCC
LIN28 - Total	F	AAGCGCAGATCAAAAGGAGA
	R	CTGATGCTCTGGCAGAAGTG
LIN28 - Endogenous	F	AGTGGCCTGGATAGGGAAGT
	R	CTTGGCTCCATGAATCTGGT
NANOG - Total	F	CAACATCCTGAACCTCAGCTAC
	R	ATTGTTCCAGGTCTGGTTGC
NANOG - Endogenous	F	TTTGGAAGCTGCTGGGGAAG
	R	GATGGGAGGAGGGGAGAGGA
GAPDH	F	GTGGACCTGACCTGCCGTCT
	R	CTGTAGCCAAATTCGTTGTC

Online Supplementary Table S2. Analysis of stem cell and differentiation markers in hiPSC (FD136-25) in normal culture conditions and after differentiation in embryoid bodies (FD136-25 hEB). Stem cell and differentiation marker expression was investigated with TaqMan® Human Stem Cell Pluripotency Array (Applied Biosystems). Results were analyzed using the comparative ΔΔCt method with *GAPDH* as the endogenous control and a normal hESC sample as calibrator (HUES-24 line; kindly provided by M. Pucéat). ND: not detected. Genes showing at least a 2-fold increase/decrease in differentiating cells compared to the undifferentiated state were considered up- or down-regulated, respectively. The formation of embryoid bodies allows a rapid assessment of pluripotent cell lines. Results from TaqMan low density arrays show a clear activation of a wide panel of genes characteristic of all three germ layers, demonstrating pluripotency of adult-derived hiPSC (blood, bone, cardiac muscle, cartilage, endothelium, muscle: mesoderm; dopaminergic neurons, motor neurons, neurons, neural, astrocytes, oligodendrocytes: ectoderm; hepatocytes, pancreas, visceral endoderm: endoderm).

Expression profile	Gene	FD136-25	FD136-25 hEB
Astrocytes	GFAP-Hs00157674_m1	0,18	0,05
Blood	HBB-Hs00747223_g1	ND	ND
Blood	HBZ-Hs00744391_s1	2,49	17,08
Bone	COL1A1-Hs00164004_m1	0,19	7,52
Bone	RUNX2-Hs00231692_m1	32,98	328,65
Cardiac Muscle	ACTC-Hs00606316_m1	0,34	0,78
Cardiac Muscle	NPPA-Hs00383230_g1	ND	24,57
Cartilage	COL2A1-Hs00156568_m1	1,71	65,68
Dopamanergic Neurons	TH-Hs00165941_m1	ND	13,52
Endogenous Control	18S-Hs99999901_s1	1,73	2,11
Endogenous Control	ACTB-Hs99999903_m1	0,77	2,00
Endogenous Control	CTNNB1-Hs00170025_m1	0,37	1,42
Endogenous Control	EEF1A1-Hs00742749_s1	0,50	0,50
Endogenous Control	GAPD-Hs99999905_m1	1,00	1,00
Endogenous Control	RAF1-Hs00234119_m1	0,32	0,57
Endothelial	CD34-Hs00156373_m1	0,26	8,00
Endothelial	CDH5-Hs00174344_m1	0,40	259,89
Endothelial	FLT1-Hs00176573_m1	1,73	3,11
Endothelial	PECAM1-Hs00169777_m1	0,27	45,01
Extraembryonic endoderm	FOXA2-Hs00232764_m1	0,09	0,38
Extraembryonic endoderm	GATA4-Hs00171403_m1	0,15	0,91
Extraembryonic endoderm	PTF1A-Hs00603586_g1	ND	ND
Germ Cell	DDX4-Hs00251859_m1	ND	ND
Germ Cell	SYCP3-Hs00538143_m1	8,18	ND
Hepatocytes	TAT-Hs00356930_m1	2,44	5,06
Mesoderm	GATA6-Hs00232018_m1	0,03	0,98
Mesoderm	T-Hs00610080_m1	0,08	1,73
Mesoderm	WT1-Hs00240913_m1	ND	25,31
Motor Neuron	HLXB9-Hs00232128_m1	0,03	0,55
Muscle	DES-Hs00157258_m1	0,26	0,69
Muscle	MYF5-Hs00271574_m1	1,77	ND
Muscle	MYOD1-Hs00159528_m1	ND	ND
Neural	NES-Hs00707120_s1	0,39	1,14
Neural	NEUROD1-Hs00159598_m1	4,68	34,09
Neural	PAX6-Hs00240871_m1	1,28	3,05
Neurons	SYP-Hs00300531_m1	0,58	0,33
Oligodendrocytes	OLIG2-Hs00377820_m1	11,98	62,78
Pancreas	GCG-Hs00174967_m1	ND	2,33
Pancreas	IAPP-Hs00169095_m1	ND	ND
Pancreas	INS-Hs00355773_m1	ND	ND
Pancreas	IPF1-Hs00236830_m1	ND	ND
Pancreas	PAX4-Hs00173014_m1	ND	ND
Pancreas	SST-Hs00174949_m1	5,05	808,59
Parietal Endoderm	FN1-Hs00277509_m1	0,40	7,20
Parietal Endoderm	LAMA1-Hs00300550_m1	0,40	2,56
Parietal Endoderm	LAMB1-Hs00158620_m1	0,24	2,14
Parietal Endoderm	LAMC1-Hs00267056_m1	0,51	2,37
Parietal Endoderm	SOX17-Hs00751752_s1	0,08	0,49

Expression profile	Gene	FD136-25	FD136-25 hEB
Stem Cell	BRIX-Hs00217848_m1	0,30	0,41
Stem Cell	CD9-Hs00233521_m1	0,12	0,09
Stem Cell	COMMD3-Hs00201350_m1	0,42	3,93
Stem Cell	CRABP2-Hs00275636_m1	1,22	7,25
Stem Cell	DNMT3B-Hs00171876_m1	0,21	0,02
Stem Cell	EBAF-Hs00745761_s1	0,00	0,00
Stem Cell	FGF4-Hs00173564_m1	0,15	0,07
Stem Cell	FGF5-Hs00170454_m1	4,23	51,08
Stem Cell	FOXD3-Hs00255287_s1	0,16	0,02
Stem Cell	GABRB3-Hs00241459_m1	0,24	0,04
Stem Cell	GAL-Hs00544355_m1	0,02	0,01
Stem Cell	GBX2-Hs00230965_m1	0,19	0,65
Stem Cell	GDF3-Hs00220998_m1	0,13	0,04
Stem Cell	GRB7-Hs00917999_g1	0,25	0,14
Stem cell	IFITM1-Hs00705137_s1	0,16	0,07
Stem Cell	IFITM2-Hs00829485_sH	0,10	0,67
Stem Cell	IL6ST-Hs00174360_m1	0,29	19,68
Stem Cell	IMP2-Hs00538956_m1	0,65	1,07
Stem Cell	KIT-Hs00174029_m1	0,26	0,45
Stem Cell	LEFTB-Hs00764128_s1	0,01	0,00
Stem Cell	LIFR-Hs00158730_m1	0,17	0,79
Stem Cell	LIN28-Hs00702808_s1	0,93	0,42
Stem Cell	Nanog*-Hs02387400_g1	0,19	0,03
Stem Cell	NODAL-Hs00415443_m1	0,07	0,04
Stem Cell	NOG-Hs00271352_s1	0,08	0,96
Stem Cell	NR5A2-Hs00187067_m1	0,15	0,61
Stem Cell	NR6A1-Hs00265966_m1	0,60	0,35
Stem Cell	PODXL-Hs00193638_m1	0,49	0,23
Stem Cell	POU5F1-Hs00742896_s1	0,66	0,03
Stem Cell	PTEN-Hs00829813_s1	0,01	0,07
Stem Cell	REST-Hs00194498_m1	0,73	0,72
Stem Cell	SEMA3A-Hs00173810_m1	0,41	0,43
Stem Cell	SFRP2-Hs00293258_m1	0,29	0,37
Stem Cell	SOX2-Hs00602736_s1	1,10	0,41
Stem Cell	TDGF1-Hs02339499_g1	0,17	0,00
Stem Cell	TERT-Hs00162669_m1	0,39	0,12
Stem Cell	TFCP2L1-Hs00232708_m1	0,18	0,18
Stem Cell	UTF1-Hs00747497_g1	0,02	0,02
Stem Cell	Xist-Hs01079824_m1	1,92	5,76
Stem Cell	ZFP42-Hs00399279_m1	0,04	0,00
Trophoblast	CDX2-Hs00230919_m1	10,81	1729,46
Trophoblast	CGB-Hs00361224_gH	0,11	1,48
Trophoblast	EOMES-Hs00172872_m1	0,24	0,11
Trophoblast	GCM1-Hs00172692_m1	ND	28,87
Trophoblast	KRT1-Hs00196158_m1	ND	ND
V3 Interneurons	ISL1-Hs00158126_m1	0,53	32,75
Visceral Endoderm	AFP-Hs00173490_m1	156,57	123419,69
Visceral Endoderm	SERPINA1-Hs00165475_m1	3,57	1532,01