Replicative senescence-associated gene expression changes in mesenchymal stromal cells are similar under different culture conditions

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Supplementary Methods

Isolation of mesenchymal stromal cells cultured with fetal bovine serum or pooled human platelet lysate

Bone marrow aspirates (maximum 13.5 mL) were obtained in 5 mL syringes preloaded with 500 IU of preservative-free heparin (Biochrom AG, Berlin, Germany) in 2.5 mL aliquots from healthy donors (n=3; aged 9, 27 and 36 years; one female, two males) after receiving written informed consent according to protocols approved by an institutional review board. After harvesting, bone marrow samples were diluted in medium without further manipulation. Cells were cultured in alpha-modified minimum essential medium (α-MEM, M4526; Sigma-Aldrich; St. Louis, MO, USA) supplemented with 25 mM HEPES (Sigma), 2 mM L-glutamine (Sigma), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco Cell Culture, Invitrogen Corporation, Grand Island, NY, USA), and either 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA) or 10% pooled human platelet lysate (pHPL). pHPL was prepared from buffy coat-derived platelet rich plasma from at least 40 whole blood donations as previously described.[1] Preservative-free heparin (2 U/mL; Biochrom AG, Berlin, Germany) was added to the medium before pHPL supplementation to avoid coagulation. The bone marrow mononuclear cell count was determined by an automated blood counter (Coulter Onyx; Beckman Coulter, Fullerton, CA, USA) and cells were seeded at a density of 0.6 – 1.0×10^4 mononuclear cells/cm^2 in three to five tissue culture flasks (Corning Inc., Acton, MA, USA) and then cultured at 37°C in 5% CO$_2$, 95% air humidity. Non-adherent cells were removed by a complete change of medium after 2 – 3 days. Twice weekly, 30% of the medium was replaced by fresh supplemented medium and cells were harvested before reaching confluence (between day 11 and 16) with 0.05% trypsin/0.7mM EDTA (1 – 5 min, 37°C; Sigma-Aldrich). Numbers of nucleated cells were determined as the mean of four measurements, using a hemocytometer. Fibroblast colony-forming units (CFU-F) were determined and cell cultures documented as described previously.[2-3] The primary culture is equivalent to passage zero (P0) and was used as the reference for senescence-associated changes.

Large scale expansion of mesenchymal stromal cells cultured with fetal bovine serum or pooled human platelet lysate

MSC derived from primary culture (P0) were seeded in α-MEM/10% pHPL and α-MEM/10% FBS with a seeding density of 30/cm^2 on 1.0 to 2.5 m^2 culture area in four to ten four-layered cell factories (CF-4; Nalge Nunc International, Naperville, IL, USA) and cultured to reach confluence. MSC from donor A were primarily cultured only in FBS-medium but were re-seeded in FBS and pHPL for P1. Partial (30%) medium change was performed twice weekly and MSC were harvested at days 12 to 13 by trypsinization (passage 1; P1). MSC derived from P1 were immediately re-seeded in α-MEM/10% pHPL and α-MEM/10% FBS at a density of 10-30 cells per cm^2 in three to four tissue culture flasks (Corning Inc., Acton, MA, USA) corresponding to a culture area of 675 – 900 cm^2 and cultured at 37°C in 5% CO$_2$, 95% air humidity for 12 to 14 days until reaching confluence (passage 2; P2). Passaging of the cells was always performed at the time when the cells formed a confluent layer. MSC were harvested with 0.05% trypsin/0.7 mM EDTA (1 – 5 min, 37°C; Sigma-Aldrich) and counted as described previously. Cumulative population doublings (PD) were calculated as described[4] in relation to the initial CFU-F frequency. Results are shown as mean ± standard error of mean, unless otherwise stated.

Isolation and expansion of mesenchymal stromal cells cultured with M1 culture medium

For comparison we used MSC that had been isolated in culture medium M1 as described earlier.[5-6] Cells were isolated from bone marrow aspirates from healthy donors after written informed consent according to guidelines approved by the Ethic Committee on the Use of Human Subjects at the University of Heidelberg. The M1 medium consisted of 58% Dulbecco's modified Eagles medium - low glucose (DMEM-LG, Cambrex, Apen, Germany) and 40% MCDB201 (Sigma, Deisenhofen, Germany), 2% FBS (HyClone, Bonn, Germany), supplemented with 2 mM L-glutamine, 100 U/mL Pen/Strep (Cambrex), 1% insulin transferrin selenium, 1% linoleic acid bovine serum albumin, 10 nM dexamethasone, 0.1 mM L-ascorbic-acid-2-phosphate (Sigma, Hamburg, Germany), PDGF-BB and EGF (10 ng/mL each, R&D Systems, Wiesbaden, Germany).[6-8] Tissue culture flasks were coated with 10 ng/mL fibronectin (Sigma) before use. MSC-M1 were always harvested upon sub-confluent growth at a density of 70% and re-plated at 10^4 cells per cm^2. Expansion was performed by the same operator throughout long-term culture to ensure similar cell densities. Seeding of the cells in numbers per cm^2 and repeated cell passages at 70% confluence resulted in a more constant cell density throughout culture-expansion.

Morphological analysis of mesenchymal stromal cells

The morphological features of MSC expanded in FBS- and pHPL-driven cultures in the early stage (maximal 12 PD) of proliferation in comparison to late passages (more than 38 cumulative PD) were analyzed by phase contrast microscopy (Olympus IX51 microscope, equipped with a COLOR-VIEW III camera and ANALYSIS B software, Olympus, Center Valley, PA, USA).

Immunophenotypic analysis of mesenchymal stromal cells cultured with fetal bovine serum or pooled human platelet lysate

The immunophenotype of FBS-MSC and pHPL-MSC was characterized after washing and blocking with sheep serum. Cells were incubated for 25 min at 4°C at different concentrations according to individual titration with fluorochrome-labeled BS-1 lectin and monoclonal antibodies against CD5, CD10, CD13, CD14, CD29, CD31, CD34, CD45, CD56, CD73, CD90 (Becton Dickinson [BD], Franklin Lakes, NJ, USA), CD105 (Caltag Laboratories; Burlingame, CA, USA), CD146 (clone P1H12; Chemicon International, Temecula, CA), HLA-AB (Harlan Sera-Lab; Leicestershire, UK) and HLA-DR (BD). As negative controls we used appropriate isotype-matched antibodies (BD). Analysis was performed with a four-color FACSCalibur® equipped with a 488 nm argon ion laser and a 635 nm red diode laser (BD).[2] Multicolor measurements were performed and data from a minimum of 10,000 viable propidium iodide-excluding cells were stored. List mode files were analyzed with CellQuest™ Pro and Paint-A-Gate Pro® software (BD). The immunophenotype of MSC-M1 was also characterized by flow cytometry as described elsewhere.[5-6]

Differentiation assays

The *in vitro* differentiation capacity of FBS-MSC, pHPL-MSC and MSC-M1 was tested: osteogenic differentiation was determined by visualization with alizarin red staining as described elsewhere,[9] whereas adipogenic differentiation was induced by the addition of insulin and identified by subsequent Oil Red O staining.[10]

References

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Online Supplementary Table S1. Primer list. Quantitative RT-PCR was performed for validation of differential gene expression in early and late passages; primers were obtained from Biospring (Frankfurt, Germany).

Gene	Amplicon length (bp)	Forward primer	Reverse primer
GAPDH	142	TTCGTCATGGGTGTGAACCA	CTGTGGTCATGAGTCCTTCCA
PARG1	309	CAATGATCATGCCCAGTGCA	GATCGTGATCTGTGCCAGGA
SERPINE1	300	CTCCTCATCCACAGCTGTCA	GCCAAGGTCTTGGAGACAGA
CDKN2B	274	CCCAACTCCACCAGATAGCA	GGGATTTCCGCATCCTAGCA
NTN4	260	AAGCCAGGCTTCTATCGTGA	TCTCCGGTGATAGGGTCACA
TOLLIP	296	CACTGTGCATGATTCCGAGA	AGGTGTCTCAATGGCATGCA
BDNF	297	CCAGGTGAGAAGAGTGATGAC	ACCCTGGACGTGTACAAGTC
MCM3	300	ACATTGGGCTACAGGACTCA	TGAATGCTGCACTCACCATC
PTN	299	CAGTGAGTCATCCGTCCAGA	GCCATTCTCCACAGTCAGAC

Online Supplementary Table S2. Senescence-associated gene expression changes in FBS-MSC. SAM analysis revealed that 74 genes were significantly expressed in P1 and P2 of FBS-MSC (FDR = 5) in relation to the corresponding P0 (30 genes up-regulated, red; 44 genes down-regulated, green).

Gene_ID	RefSeq_NM	Gene_Symbol	Gene_Name
hCG39210.3	NM_001884.2	HAPLN1	hyaluronan and proteoglycan link protein 1
hCG1785142.2			
hCG21175.2	NM_020529.1	NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
hCG1786452.2	NM_032499.3	HH114	hypothetical protein HH114
hCG37565.3	NM_152549.1	MGC39633	hypothetical protein MGC39633
hCG1747552.2	NM_022917.4;	NOL6	nucleolar protein family 6 (RNA-associated)
hCG37145.3	NM_004613.2	TGM2	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)
hCG43757.2	NM_000224.2;	KRT18	keratin 18
hCG1818503.1			
hCG21529.3	NM_001196.2;	BID	BH3 interacting domain death agonist
hCG37641.4			
hCG1644263.4			
hCG2043058	NM_003607.2;	CDC42BPA	CDC42 binding protein kinase alpha (DMPK-like)
hCG26491.4	NM_001709.3;	BDNF	brain-derived neurotrophic factor
hCG37727.3	NM_005723.2	TM4SF9	transmembrane 4 superfamily member 9
hCG29950.3	NM_018946.2	NANS	N-acetylneuraminic acid synthase (sialic acid synthase)
hCG1786812.3			
hCG2030654			
hCG2039077			
hCG41325.3	NM_015395.1	DKFZP434B03	DKFZP434B0335 protein
hCG34035.3	NM_000636.1	SOD2	superoxide dismutase 2, mitochondrial
hCG41855.3	NM_007032.3;	HRIHFB2122	Tara-like protein
hCG29298.3	NM_002982.2	CCL2	chemokine (C-C motif) ligand 2
hCG15194.3			
hCG1778706.1			
hCG2029617	NM_021135.3	RPS6KA2	ribosomal protein S6 kinase, 90kDa, polypeptide 2
hCG32035.3	NM_004815.2	PARG1	PTPL1-associated RhoGAP 1
hCG20724.3	NM_012099.1	ASE-1	CD3-epsilon-associated protein; antisense to ERCC-1
hCG22888.3	NM_014226.1	RAGE	renal tumor antigen
hCG1743404.1	NM_024810.1	FLJ23018	hypothetical protein FLJ23018
hCG15495.3	NM_003014.2	SFRP4	secreted frizzled-related protein 4
hCG2043616	NM_130830.2	LRRC15	leucine rich repeat containing 15
hCG28158.2	NM_000877.2	IL1R1	interleukin 1 receptor, type I
hCG24961.2	NM_020375.1	C12orf5	chromosome 12 open reading frame 5
AF208161.1		ERVWE1	endogenous retroviral family W, env(C7), member 1 (syncytin)
hCG26534.3	NM_006982.1	CART1	cartilage paired-class homeoprotein 1
hCG38642.3	NM_020190.1	OLFML3	olfactomedin-like 3
hCG1995175			
hCG1799751.2			
hCG27360.2	NM_032590.2	FBXL10	F-box and leucine-rich repeat protein 10
hCG2040309			
hCG2042157			
hCG18946.3	NM_002825.5	PTN	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)
hCG2009388	NM_006209.2	ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)
hCG401151.3	NM_002040.2	GABPA	GA binding protein transcription factor, alpha subunit 60kDa
hCG1642637.3			
hCG1990594.1	NM_001187.1;	BAGE;BAGE2;	B melanoma antigen;B melanoma antigen family, member 2;B melanoma antigen family, member 3; B melanoma antigen family, member 4;B melanoma antigen family, member 5;myeloid/lymphoid or mixed-lineage leukemia 3
hCG2038907	NM_021631.1	FKSG2	apoptosis inhibitor
hCG1987903	NM_032287.1	DKFZp761O17	hypothetical protein DKFZp761O17121
hCG16680.4	NM_017549.1	UCC1	upregulated in colorectal cancer gene 1
hCG39580.3	NM_003711.2;	PPAP2A	phosphatidic acid phosphatase type 2A
hCG401152.3	NM_021219.2	JAM2	junctional adhesion molecule 2
hCG23456.3	NM_024336.1	IRX3	iroquois homeobox protein 3
hCG1993006.1	NM_207422.1	FLJ44635	FLJ44635 protein
hCG1641962.2	NM_020813.1	ZNF471	zinc finger protein 471
hCG1995870	NM_022834.3;	WARP	von Willebrand factor A domain-related protein
hCG1640501.2			
hCG22389.3			
hCG2039436.1	NM_002751.5	MAPK11	mitogen-activated protein kinase 11
hCG38036.3	NM_030762.1	BHLHB3	basic helix-loop-helix domain containing, class B, 3
hCG32877.3	NM_018322.1	C6orf64	chromosome 6 open reading frame 64
hCG1806413.2			
hCG2039382			
hCG20517.1	NM_005737.3	ARL7	ADP-ribosylation factor-like 7
hCG14635.2	NM_004202.2	TMSB4Y	thymosin, beta 4, Y-linked
hCG37398.3	NM_005900.1;	SMAD1	SMAD, mothers against DPP homolog 1 (Drosophila)
hCG1641231.3	NM_005853.4	IRX5	iroquois homeobox protein 5
hCG1820437.1			
hCG15738.3	NM_003387.3	WASPIP	Wiskott-Aldrich syndrome protein interacting protein
hCG1744836.2		MGC16291	hypothetical protein MGC16291
hCG2006491	NM_014795.2	ZFHX1B	zinc finger homeobox 1b
hCG25390.3	NM_130783.2	LOC90139	tetraspanin similiar to uroplakin 1
hCG1741134.1	NM_003641.2	IFITM1	interferon induced transmembrane protein 1 (9-27)
hCG1737352.2	NM_000314.2	PTEN	phosphatase and tensin homolog (mutated in multiple advanced cancers 1)

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Online Supplementary Table S3. Senescence-associated gene expression changes in pHPL-MSC. In P1 and P2 of pHPL-MSC, SAM analysis revealed that 227 genes were significantly expressed (FDR = 5) in relation to the corresponding P0 (68 genes up-regulated, red; 159 genes down-regulated, green).[2]

Gene_ID	RefSeq_NM	Gene_Symbol	Gene_Name
hCG16678.2			
hCG42844.3	NM_020371.1	AVEN	apoptosis, caspase activation inhibitor
hCG28722.3	NM_033375.3	MYO1C	myosin IC
hCG39086.3	NM_015641.2;	TES	testis derived transcript (3 LIM domains)
hCG24063.3	NM_144573.1	NEXN	nexilin (F actin binding protein)
NM_022791.2	NM_002429.3;	MMP19	matrix metalloproteinase 19
hCG1784765.1	NM_007216.3;	HPS5	Hermansky-Pudlak syndrome 5
hCG1644625.2			
hCG1648323.2	NM_207329.1	MYADML	myeloid-associated differentiation marker-like
hCG30297.2	NM_002087.1	GRN	granulin
hCG23583.3	NM_015526.1	CLIPR-59	CLIP-170-related protein
hCG1813648.1			
hCG2042185.1			
hCG33052.4	NM_032515.3	BOK	BCL2-related ovarian killer
hCG17155.3	NM_005578.1	LPP	LIM domain containing preferred translocation partner in lipoma
hCG15041.3	NM_003506.2	FZD6	frizzled homolog 6 (Drosophila)
hCG23105.3	NM_000604.2;	FGFR1	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)
hCG1820806.1	NM_032862.2	TIGD5	tigger transposable element derived 5
hCG19834.5			
hCG1790447.2			
hCG25059.3	NM_000302.2	PLOD	procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase, Ehlers-Danlos syndrome type VI)
hCG401305.3	NM_000211.1	ITGB2	integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage antigen 1 (mac-1) beta subunit)
hCG2030654			
hCG15683.3	NM_002568.2	PABPC1	poly(A) binding protein, cytoplasmic 1
hCG42857.3	NM_033557.1	LOC90522	similar to putative transmembrane protein; homolog of yeast Golgi membrane protein Yif1p (Yip1p-interacting factor)
hCG2011004	NM_000268.2;	NF2	neurofibromin 2 (bilateral acoustic neuroma)
hCG1817523.2			
hCG1779807.4	NM_152753.2	SCUBE3	signal peptide, CUB domain, EGF-like 3
hCG38502.3	NM_001672.2	ASIP	agouti signaling protein, nonagouti homolog (mouse)
hCG1643175.3			
hCG2029617	NM_021135.3	RPS6KA2	ribosomal protein S6 kinase, 90kDa, polypeptide 2
hCG17829.4	NM_022121.2	PERP	PERP, TP53 apoptosis effector
hCG37261.2			
hCG28342.3			
hCG33090.3			
hCG24047.3	NM_013943.1	CLIC4	chloride intracellular channel 4
hCG43757.2	NM_000224.2;	KRT18	keratin 18
hCG1743768.2	NM_006945.2	SPRR2B	small proline-rich protein 2B
hCG32035.3	NM_004815.2	PARG1	PTPL1-associated RhoGAP 1
ENST00000344368			
hCG20745.2	NM_002206.1	ITGA7	integrin, alpha 7
hCG2040540			
hCG32885.3	NM_017949.1	CUEDC1	CUE domain containing 1
hCG1640983.2	NM_003524.2	HIST1H2BH	histone 1, H2bh
hCG2007564.1			
hCG38966.3	NM_024111.2	MGC4504	hypothetical protein MGC4504
hCG1818503.1			
hCG201364.3	NM_130786.2	A1BG	alpha-1-B glycoprotein
hCG22650.3			
hCG39210.3	NM_001884.2	HAPLN1	hyaluronan and proteoglycan link protein 1
hCG15194.3			
hCG37191.2	NM_005257.3	GATA6	GATA binding protein 6
hCG1736101.3			
hCG1988320			
hCG22888.3	NM_014226.1	RAGE	renal tumor antigen
hCG2005139.1	NM_080593.1	HIST1H2BK	histone 1, H2bk
hCG1642235.3			
hCG1786812.3			
hCG1770271.2			
hCG26491.4	NM_001709.3;	BDNF	brain-derived neurotrophic factor
hCG1643885.4			
hCG1787353.1	NM_003523.2	HIST1H2BE	histone 1, H2be
hCG1644263.4			
hCG25627.5			
hCG1785142.2			
hCG27464.3	NM_020679.2	AD023	AD023 protein
hCG17353.3	NM_000602.1	SERPINE1	serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1
hCG28076.2	NM_018689.1	KIAA1199	KIAA1199 protein
hCG39157.3	NM_016343.2	CENPF	centromere protein F, 350/400ka (mitosin)
hCG2024276	NM_007057.2;	ZWINT	ZW10 interactor
hCG1785851.2	NM_005192.2	CDKN3	cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)
hCG40929.2	NM_022346.2	HCAP-G	chromosome condensation protein G
hCG31373.3	NM_014791.2	MELK	maternal embryonic leucine zipper kinase
hCG39940.2	NM_005733.1	KIF20A	kinesin family member 20A
hCG17257.3	NM_002380.2;	MATN2	matrilin 2
hCG1998251	NM_032032.1	FKSG42	FKSG42
hCG1810802.1			
hCG38372.2	NM_007019.2;	UBE2C	ubiquitin-conjugating enzyme E2C
hCG1640822.3	NM_003095.1	SNRPF	small nuclear ribonucleoprotein polypeptide F
hCG32579.3	NM_003981.2;	PRC1	protein regulator of cytokinesis 1
hCG38821.3	NM_012112.4	TPX2	TPX2, microtubule-associated protein homolog (Xenopus laevis)
hCG1999761.1	NM_052852.1		
hCG17224.2	NM_005496.2	SMC4L1	SMC4 structural maintenance of chromosomes 4-like 1 (yeast)

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hCG1743861.1			
hCG1820431.1			
hCG1776116.2		KIF2C	kinesin family member 2C
hCG38853.3	NM_016359.1;	NUSAP1	nucleolar and spindle associated protein 1
hCG28645.3	NM_018193.1	FLJ10719	hypothetical protein FLJ10719
hCG27811.2	NM_001168.1	BIRC5	baculoviral IAP repeat-containing 5 (survivin)
hCG2043508			
hCG20202.4	NM_013277.2	RACGAP1	Rac GTPase activating protein 1
hCG401183.3	NM_005111.5;	CRYZL1;DON	crystallin, zeta (quinone reductase)-like 1;downstream neighbor of SON
hCG39533.3	NM_018131.3	C10orf3	chromosome 10 open reading frame 3
hCG1813144.1	NM_001067.2	TOP2A	topoisomerase (DNA) II alpha 170kDa
hCG1734395.2			
hCG1739274.2	NM_001826.1	CKS1B	CDC28 protein kinase regulatory subunit 1B
hCG38762.3	NM_020183.3	ARNTL2	aryl hydrocarbon receptor nuclear translocator-like 2
hCG37510.4	NM_015895.3	GMNN	geminin, DNA replication inhibitor
hCG38973.3			
hCG40242.3	NM_001786.2;	CDC2	cell division cycle 2, G1 to S and G2 to M
hCG1818259.2	NM_017975.2	FLJ10036	hypothetical protein FLJ10036
hCG1734075.2			
hCG37423.2	NM_014783.2	ARHGAP11A	similar to human GTPase-activating protein
hCG39252.4	NM_004358.3;	CDC25B	cell division cycle 25B
hCG22621.3	NM_003380.1	VIM	vimentin
hCG39238.3	NM_005342.1	HMGB3	high-mobility group box 3
hCG37306.3	NM_005124.2	NUP153	nucleoporin 153kDa
hCG40513.3	NM_002129.2	HMGB2	high-mobility group box 2
hCG17376.2			
hCG17407.4	NM_002947.2	RPA3	replication protein A3, 14kDa
hCG1776859.2	NM_016058.1	CGI-121	CGI-121 protein
hCG1757337.2	NM_000859.1	HMGCR	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
hCG1648136.3			
hCG23744.3	NM_016195.2	MPHOSPH1	M-phase phosphoprotein 1
hCG41525.3	NM_006739.2	MCM5	MCM5 minichromosome maintenance deficient 5, cell division cycle 46 (S. cerevisiae)
hCG39269.2	NM_004526.2	MCM2	MCM2 minichromosome maintenance deficient 2, mitotin (S. cerevisiae)
hCG1739274.2	NM_001826.1	CKS1B	CDC28 protein kinase regulatory subunit 1B
hCG1787380.1	NM_003542.3	HIST1H4C	histone 1, H4c
hCG2041364.1			
hCG2012475	NM_017718.1		
hCG96817.3			
hCG27811.2	NM_001168.1	BIRC5	baculoviral IAP repeat-containing 5 (survivin)
hCG39396.2	NM_002105.1	H2AFX	H2A histone family, member X
hCG39720.3	NM_003878.1	GGH	gamma-glutamyl hydrolase (conjugase, folylpolygammaglutamyl hydrolase)
hCG1817522.1			
hCG1812088.2			
hCG40880.3	NM_002482.2;	NASP	nuclear autoantigenic sperm protein (histone-binding)
hCG40513.3	NM_002129.2	HMGB2	high-mobility group box 2
hCG2027327			
hCG1996013			
hCG1787686.2			
hCG39922.3	NM_032636.4	DDA3	differential display and activated by p53
hCG14802.2	NM_003017.3	SFRS3	splicing factor, arginine/serine-rich 3
hCG22498.3	NM_002388.3	MCM3	MCM3 minichromosome maintenance deficient 3 (S. cerevisiae)
hCG2030503.1			
hCG15962.3	NM_019063.2	EML4	echinoderm microtubule associated protein like 4
hCG27392.4	NM_018490.1	GPR48	G protein-coupled receptor 48
hCG1820748.1			
hCG2016110	NM_032290.1	ANKRD32	ankyrin repeat domain 32
hCG39757.3	NM_003146.2	SSRP1	structure specific recognition protein 1
hCG2032518	NM_005517.1	HMGN2	high-mobility group nucleosomal binding domain 2
hCG1811567.1	NM_018098.4	ECT2	epithelial cell transforming sequence 2 oncogene
hCG2032518	NM_005517.1	HMGN2	high-mobility group nucleosomal binding domain 2
hCG2039386	NM_014736.3	KIAA0101	KIAA0101
hCG41133.3			
hCG27173.3	NM_031966.2	CCNB1	cyclin B1
hCG1759545.1	NM_000791.2	DHFR	dihydrofolate reductase
hCG1985268	NM_012227.1	PGPL	pseudoautosomal GTP-binding protein-like
hCG41089.3	NM_012399.2	PITPNB	phosphotidylinositol transfer protein, beta
hCG1790765.2			
hCG23474.2	NM_002497.1	NEK2	NIMA (never in mitosis gene a)-related kinase 2
hCG1641980.5	NM_005542.3;	INSIG1	insulin induced gene 1
hCG19812.3	NM_006579.1	EBP	emopamil binding protein (sterol isomerase)
hCG17322.3	NM_020548.2	DBI	diazepam binding inhibitor (GABA receptor modulator, acyl-Coenzyme A binding protein)
hCG1812753.2	NM_018071.2		
hCG1999834	NM_002828.2;	PTPN2	protein tyrosine phosphatase, non-receptor type 2
hCG2005852.1			
hCG1818621.1			
hCG39163.4	NM_003686.3;	EXO1	exonuclease 1
hCG1984888			
AK125429.1			
hCG2032518	NM_005517.1	HMGN2	high-mobility group nucleosomal binding domain 2
hCG2020366	NM_002128.3	HMGB1	high-mobility group box 1
hCG1644442.2			
hCG1992641			
hCG1785387.1			
hCG1994842.1		TYMS	thymidylate synthetase

to be continued in the next page

hCG32517.5		COL27A1	collagen, type XXVII, alpha 1
hCG41550.3	NM_024053.2	C22orf18	chromosome 22 open reading frame 18
hCG96156.1	NM_005520.1	HNRPH1	heterogeneous nuclear ribonucleoprotein H1 (H)
hCG18946.3	NM_002825.5	PTN	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)
hCG23164.3	NM_001255.1	CDC20	CDC20 cell division cycle 20 homolog (S. cerevisiae)
hCG1642740.3			
hCG2023664.1	NM_006716.3	ASK	activator of S phase kinase
hCG1739274.2	NM_001826.1	CKS1B	CDC28 protein kinase regulatory subunit 1B
hCG17108.3	NM_021105.1	PLSCR1	phospholipid scramblase 1
hCG17616.2	NM_003372.3	VBP1	von Hippel-Lindau binding protein 1
hCG21668.3	NM_001889.2	CRYZ	crystallin, zeta (quinone reductase)
hCG2014421			
hCG15915.3			
hCG19828.3	NM_002668.1	PLP2	proteolipid protein 2 (colonic epithelium-enriched)
hCG23785.3	NM_004671.1;	PIAS2	protein inhibitor of activated STAT, 2
hCG15521.3			
hCG1811039.1	NM_080654.1	NY-REN-41	NY-REN-41 antigen
hCG2040289			
hCG20839.2	NM_007029.2	STMN2	stathmin-like 2
hCG2039660.1	NM_002266.1	KPNA2	karyopherin alpha 2 (RAG cohort 1, importin alpha 1)
hCG1995887.1			
hCG1812037.1	NM_004442.4;	EPHB2	EphB2
hCG1644309.4			
hCG26513.4	NM_006851.1	GLIPR1	GLI pathogenesis-related 1 (glioma)
hCG1743134.2	NM_018204.2	CKAP2	cytoskeleton associated protein 2
hCG34031.2	NM_005891.1	ACAT2	acetyl-Coenzyme A acetyltransferase 2 (acetoacetyl Coenzyme A thiolase)
hCG2014578	NM_007192.2	SUPT16H	suppressor of Ty 16 homolog (S. cerevisiae)
hCG2032518	NM_005517.1	HMGN2	high-mobility group nucleosomal binding domain 2
hCG19242.3	NM_012412.3;	H2AFV	H2A histone family, member V
hCG29352.3	NM_002146.3	HOXB3	homeo box B3
hCG27344.3			
hCG39115.3	NM_002592.2;	PCNA	proliferating cell nuclear antigen
hCG28547.3	NM_016397.2;	TH1L	TH1-like (Drosophila)
hCG20885.3	NM_033402.2	KIAA1764	KIAA1764 protein
hCG2041550.1			
hCG22733.3		DEPDC1	DEP domain containing 1
hCG20166.2	NM_004848.1	C1orf38	chromosome 1 open reading frame 38
hCG16911.2	NM_004237.2	TRIP13	thyroid hormone receptor interactor 13
hCG40848.3	NM_004111.4	FEN1	flap structure-specific endonuclease 1
hCG1999411			
hCG40606.3	NM_024834.1	C10orf119	chromosome 10 open reading frame 119
hCG24065.3	NM_006417.2	IFI44	interferon-induced protein 44
hCG2042151			
hCG1787379.1	NM_003544.2	HIST1H4B	histone 1, H4b
hCG2009021	NM_014109.2	ATAD2	ATPase family, AAA domain containing 2
hCG1983999			
hCG2007731			
hCG41784.3	NM_001948.2	DUT	dUTP pyrophosphatase
hCG2032518	NM_005517.1	HMGN2	high-mobility group nucleosomal binding domain 2
hCG1750237.3			
hCG1750332.2			
hCG16307.3	NM_004219.2	PTTG1	pituitary tumor-transforming 1
hCG2020860			
hCG17108.3	NM_021105.1	PLSCR1	phospholipid scramblase 1
hCG16461.2	NM_000788.1	DCK	deoxycytidine kinase
hCG2020693	NM_016271.3;	RNF138	ring finger protein 138
hCG33379.2	NM_004645.1	COIL	coilin
hCG16827.4			
hCG31640.2	NM_002540.3;	ODF2	outer dense fiber of sperm tails 2
hCG172489.3	NM_006397.2	RNASEH2A	ribonuclease H2, large subunit

Online Supplementary Table S4. Chromosomal location of differentially expressed genes. The representation of the differentially expressed genes within chromosomal regions was analyzed.

	FBS			pHPL			MSC-M1	
Gene Set [N]	#	*P* value	Gene Set [N]	#	*P* value	Gene Set [N]	#	*P* value
			chr1p35 [121]	5	6.52 e^{-3}	chr1p36 [573]	7	2.68 e^{-2}
chr2q34 [30]	2	3.67 e^{-2}	chr2q34 [30]	2	3.38 e^{-2}			
						chr2p13 [90]	6	1.23 e^{-2}
chr2p23 [105]	3	7.65 e^{-2}				chr2q22 [27]	3	1.97 e^{-2}
chr3p23 [20]	2	1.79 e^{-2}	chr3p22 [89]	3	4.95 e^{-2}	chr3p11 [10]	2	1.93 e^{-2}
						chr3q24 [31]	3	2.76 e^{-2}
chr4q31 [107]	3	7.92 e^{-2}	chr4p16 [179]	4	7.39 e^{-2}	chr4q21 [124]	9	1.58 e^{-3}
			chr4q26 [37]	2	4.81 e^{-2}			
			chr4q27 [21]	2	1.79 e^{-2}			
			chr5q32 [37]	2	4.81 e^{-2}	chr5p12 [24]	3	1.46 e^{-2}
						chr5q12 [58]	5	8.12 e^{-3}
						chr5q13 [112]	7	9.99 e^{-3}
chr6p22 [188]	5	3.6 e^{-2}	chr6p22 [188]	7	2.53 e^{-3}	chr6p22 [188]	11	2.66 e^{-3}
chr6q22 [108]	3	8.06 e^{-2}				chr6p11 [14]	2	3.56 e^{-2}
chr6q23 [80]	3	4.38 e^{-2}				chr7q31 [118]	6	3.43 e^{-2}
chr6q25 [96]	5	3.16 e^{-3}	chr6q25 [96]	3	5.8 e^{-2}			
			chr8p21 [110]	4	2.08 e^{-2}	chr8p21 [110]	6	2.68 e^{-2}
			chr8q22 [110]	4	2.08 e^{-2}	chr8q12 [57]	4	3.1 e^{-2}
chr8q24 [239]	6	2.94 e^{-2}	chr8q24 [239]	5	6.18 e^{-2}			
chr9q34 [299]	10	1.12 e^{-3}				chr9q31 [89]	5	3.62 e^{-2}
			chr11q23 [173]	5	2.36 e^{-2}	chr11p13 [62]	4	3.9 e^{-2}
chr12q23 [106]	3	7.78 e^{-2}	chr12q12 [75]	3	3.39 e^{-2}			
			chr13q34 [56]	3	1.69 e^{-2}			
chr14q22 [92]	3	5.89 e^{-2}				chr14q11 [344]	2	1.16 e^{-2}
chr14q24 [166]	4	6.98 e^{-2}						
chr14q32 [469]	2	8.2 e^{-2}						
chr15q22 [121]	4	3.14 e^{-2}				chr15q22 [121]	8	4.67 e^{-3}
chr16q11 [16]	2	1.18 e^{-2}	chr16q13 [53]	5	1.87 e^{-4}			
chr17q23 [105]	4	2.1 e^{-2}	chr17q23 [105]	3	6.94 e^{-2}			
chr18q11 [58]	4	3.07 e^{-3}				chr17q21 [354]	16	4.21 e^{-3}
			chr19q13 [1011]	13	7.98 e^{-2}	chr19q13 [1011]	8	1.59 e^{-4}
chr20q12 [53]	3	1.66 e^{-2}						
chr20q13 [264]	8	5.57 e^{-3}	chr20q13 [264]	5	7.92 e^{-2}			
			chr21q22 [307]	7	2.46 e^{-2}			

Positional information to chromosomal bands was analyzed for two-fold differentially expressed genes (up and down-regulated) in the three datasets by GSEA analysis. The number of genes per chromosomal band (N), the number of differentially expressed genes in this region (#) and the probability (P value; hypergeometric distribution) are provided.

Online Supplementary Table S5. Results of array comparative genome hybridization analysis. Copy number variations of MSC from three donors cultured in FBS and pHPL-driven medium and of three donors in M1 medium are listed according to the chromosomes and cytobands. Amplifications (in bold) or deletions, the size of the affected region and *P* values are indicated.

Donor	Passage	Chromosome	Cytoband	Aberration	kb	p-value
FBS A	Early	7	q11.22 - q11.23	-0.374435	5159	5.06E-51
		22	q11.23	-4.217975	43	2.88E-80
	Late	**2**	**q32.2 - q32.3**	**0.607917**	**370**	**2.14E-10**
		22	q11.23	-4.067567	43	9.06E-74
pHPL A	Early	n.t.	n.t.	n.t.	n.t.	n.t.
	Late	**2**	**q35**	**2.022606**	**74**	**1.05E-27**
		22	q11.23	-4.34042	43	1.80E-78
FBS B	Early	n.t.	n.t.	n.t.	n.t.	n.t.
	Late	2	q13	-0.876383	118	1.995E-11
		3	p14.2	-0.877506	150	2.326E-14
		8	**p11.23 - p11.22**	**0.764326**	**155**	**2.562E-11**
		15	q11.2	-1.325284	1301	5.923E-98
		22	q11.23	-3.582023	43	2.179E-72
pHPL B	Early	**2**	**q35**	**1.990514**	**74**	**6.273E-26**
		3	p14.2	-0.841847	150	7.21E-12
		15	q11.2	-1.399927	1301	2.09E-95
		22	q11.23	-4.166664	43	2.985E-82
	Late	2	q13	-0.910015	132	6.913E-13
		2	**q35**	**1.882569**	**74**	**8.96E-28**
		3	p14.2	-0.917076	103	5.304E-11
		11	q11	-1.080188	53	2.645E-10
		15	q11.2	-1.427194	1301	3.649E-109
		22	q11.23	-3.760139	43	5.409E-68
FBS C	Early	6	p21.33	-3.197399	42	6.763E-64
		6	**q13 - q14.1**	**0.951695**	**98**	**3.366E-12**
		8	p11.23	-4.34232	138	4.194E-187
		12	**p13.31**	**1.331197**	**76**	**1.324E-19**
		16	**p11.1**	**0.929597**	**162**	**1.808E-10**
	Late	6	p21.33	-2.764336	42	9.684E-39
		8	p11.23	-4.043672	126	1.103E-148
		12	**p13.31**	**1.462532**	**76**	**2.438E-16**
pHPL C	Early	6	p21.33	-3.060614	42	8.543E-58
		8	p11.23	-4.091248	126	1.687E-170
	Late	6	p21.33	-3.035865	42	2.889E-61
		8	p11.23	-4.564849	126	2.196E-183
		12	**p13.31**	**1.328333**	**76**	**2.775E-20**
		16	**p11.1**	**0.904776**	**162**	**2.312E-10**
MSC M1 A	Early	**2**	**q35**	**1.358999**	**74**	**2.861E-22**
		4	p15.32	-1.165403	256	2.744E-12
		8	p22	-1.076421	62	7.346E-13
		8	p11.23	-4.01017	138	5.423E-178
		15	q11.2	-0.572011	1071	6.714E-17
	Late	8	p11.23	-3.840196	138	4.023E-160
		15	q25.3	-0.670904	221	6.651E-11
MSC M1 B	Early	**2**	**q35**	**1.763953**	**74**	**2.184E-25**
		3	q29	-0.477415	532	1.304E-12
		8	**p11.23 - p11.22**	**0.901613**	**155**	**9.725E-14**
		15	q11.2	-1.030499	561	1.06E-31
	Late	**2**	**q35**	**1.979692**	**74**	**3.346E-25**
		8	**p11.23 - p11.22**	**0.921143**	**155**	**2.06E-11**
		15	q11.2	-0.994621	561	2.395E-23
MSC M1 C	Early	**2**	**q35**	**1.640943**	**74**	**1.172E-23**
		8	p23.1	-0.980526	668	2.957E-12
		9	q22.31	-0.871701	201	1.399E-15
		12	q24.33	-0.608377	389	3.173E-15
	Late	8	p23.1	-1.165842	553	6.496E-10
		9	q22.31	-0.884033	167	6.27E-12
		15	q11.2	-0.528808	1220	5.881E-13