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A matter of identity – phenotype and differentiation potential of human somatic stem cells

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Supplementary Information

Extended Methods

Isolation and culturing of neural stem cells (NSCs)

All procedures involving human tissue were carried out in accordance to the Human Tissue Act 2006. The human embryonic and fetal material was provided by the Joint MRC (grant G070089)/ Wellcome Trust (grant GR082557) Human Developmental Biology Resource (http://hdbr.org). The human neural stem cell lines (hNSC) lines were derived from brain and spinal cords from human embryos and fetuses between Carnegie stage (CS) 18 (gestation age 37–42 days) and CS22 (gestational age 54–56 days). 22). Brains and spinal cords were dissected in cold Neurobasal-A medium (Gibco, #10888022) under aseptic conditions, meninges and blood vessels were removed using fine forceps and processed essentially as described by Sun et al 2008. In brief, the dissected tissues were minced and transferred into 0.5 ml accutase solution (Stem cell pro, Gibco, #A11105-01) using a sterile Pasteur pipette and incubated at 37°C for 5-10 minutes with occasional trituration with a P1000 pipette to obtain a single cell suspension. After digestion and trituration, the cells were centrifuged at 1000rpm for 5 minutes. This consisted RHB-A medium (Stem Cell Sciences Ltd, UK), supplemented with L-glutamine (2 mM final, Invitrogen), penicillin-streptomycin (10 mL/L final, Invitrogen), N2 (1X final, PAA), B27 (1X final, PAA), recombinant human FGF (20 ng/mL final, Peprotech), recombinant human EGF (20 ng/mL final Peprotech), Heparin Sulfate (5 µg/mL final, Sigma), Laminin L2020 (10 µg/mL final, Sigma) and BSA Fraction V (100 µg/mL final, Sigma). NSCs were split 1:2 or 1:3 every 3-4 days using accutase (Invitrogen).

Isolation and culturing of umbilical cord mesechymal stem cells (UC-MSCs)

Umbilical cord tissue was supplied by the Anthony Nolan Cell Therapy Centre (http://www.anthonynolan.org/Healthcareprofessionals/Cord-blood-services.aspx) from informed consent mothers with healthy full-term pregnancies. Tissue was processed within 6 hrs of birth. The cord tissue was rinsed with 1x PBS to remove as much blood as possible and the then the blood vessels were removed. The remaining cord matrix or Wharton's Jelly was cut into much smaller pieces and placed into enzyme solution (0.5mg/ml of Hyaluronidase [Sigma] and 0.5mg/ml of Collagenase [Sigma]) and mixed on a MACS MixTM Tube Rotator (Miltenyi) at 37°C for 1 hr. 0.125% Trypsin (Sigma) was added to the tissue and it was re-placed in the tube rotator at 37°C for 30 mins. The final product was passed through 70µm cell strainer (BD Bioscience), washed with 1x PBS, and centrifuged at 300g for 5 mins at RT. The resulting pellet was treated with ammonium chloride solution (STEMCELL TECHNOLOGIES) for 5 mins to lyse remaining RBC, washed with 1x PBS, centrifuged at 300g for 5 mins, and re-suspended in appropriate culture media.

Isolation and culturing of human adipose tissue-derived stem cells (ADSCs)

ADSCs were prepared from surplus raw lipoaspirates (2-10 ml) of consented paediatric patients. Lipoaspirates were washed extensively with Phosphate-Buffered Saline (PBS, PAA Laboratories) and then incubated with two volumes of 0.05% trypsin/1mM EDTA (Life Technologies) in a 37°C shaking incubator for 1 hr. They were then centrifuged at 500 g for 5 minutes. After removing the supernatant and the floating mature adipocytes, cell pellets were incubated with red blood cell lysis buffer (Roche) at room temperature for 5 minutes and then centrifuged again. The so-called 'stromal vascular fraction' containing ASDCs was subsequently grown in proliferation medium consisting of DMEM high glucose (Life Technologies) supplemented with 10% ES-FBS (Embryonic Stem cell-qualified fetal bovine serum, Invitrogen), 2 mM glutamine and 1% penicillin/streptomycin (all from Life Technologies) at 37°C in a humidified incubator with 5% CO2.

Adipogenic differentiation - quantification

Confluent ADSCs were incubated with a medium containing DMEM 10% ES-FBS, 10 ng/ml insulin, 500 mM 3-isobutyl-1-methylxanthine (IBMX), 1 mM dexamethasone, 1 mM rosiglitazone (Molekula). After three weeks, cells were fixed with 10% formalin, washed with 60% isopropanol for 5 minutes stained with Oil-Red O (0.25% w/v) for 10 minutes. After staining, cells were washed

several times with H2O. For quantification, the dye was extracted with 100% isopropanol for 30 minutes at room temperature and OD495 determined with a plate reader (Bio-Rad).

Chondrogenic differentiation - quantification

Confluent ADSCs were incubated in a medium containing DMEM 10% ES-FBS, 0.1 µM dexamethasone, 10 ng/ml Tgfβ1 (R&D Systems), ITS (Life Technologies), 50 µg/ml ascorbate. After three weeks, cells were fixed in 4% paraformaldehyde (PFA), rinsed with 0.1N HCl for 5 minutes and stained with Alcian Blue (1% in 0.1N HCl). For quantification, the dye was extracted with guanidine hydrochloride 6M overnight at room temperature and OD595 determined.

Osteogenic differentiation - quantification

Confluent ADSCs were incubated in a medium containing DMEM 10% ES-FBS, 0.1 µM dexamethasone, 100 µg/ml ascorbate, 10 mM β-glycerophosphate. After three weeks, cells were fixed in ice-cold 70% ethanol for 1 hour, washed with H20 and stained with 1% Alizarin Red. For quantification, stained cells were incubated with 10% acetic acid for 30 minutes at room temperature, scraped, transferred to 1.5 ml vials, and heated at 85°C for 10 minutes. Debris was eliminated by centrifugation and the OD405 of the supernatants determined.

Supplemental Table 1. Primary and secondary antibodies used in this study for immunohistochemistry/ immunocytochemistry.

Antibody	**Species**	**Types**	**Dilution** [Amount of antibody]*		**Source**
			Cultured cells	Tissue	
Primary					
Vimentin	Mouse	IgG	1:100	1:200	DAKO
αSMA	Mouse	IgG	1:200 [10µg/ml]	1:100 [20µg/ml]	Abcam
Sox2	Rabbit	IgG	1:1000	1:200	Millipore
Nestin	Rabbit	IgG	1:200	1:400	Millipore
GFAP	Rabbit	IgG	1:1000	1:400	Millipore
βIII-tubulin	Mouse	IgG	1:1000 [1µg/ml]	1:400 [2.5µg/ml]	Promega
NF200	Rabbit	IgG	1:200 [40µg/ml]	1:100 [80µg/ml]	Sigma
Secondary					
anti-mouse IgG Alexa Fluor® 488	Donkey	IgG	1:400 [5µg/ml]		Molecular probe, Invitrogen
anti-rabbit IgG Alexa Fluor® 488	Donkey	IgG	1:400 [5µg/ml]		Molecular probe, Invitrogen

*The amount of antibody used has been indicated when available.

Supplemental Table 2. Conjugated antibodies used in this study for flow cytometry.

Name	Fluorochrome	Clone	Isotype	Dilution	Company
Anti-GFAP	PE	1B4	IgG2b	1:20	BD Pharmingen
Anti-MAP2B	AlexaFluor® 488	18/MAP2B	IgG1, k	1:20	BD Pharmingen
Anti-Nestin	PE	25/NESTIN	IgG1, k	1:20	BD Pharmingen
Anti-O4	PE	O4	IgM	1:10	R&D Systems
Anti-Oct3/4	PerCP-Cy 5.5	40/Oct-3	IgG1, k	1:20	BD Pharmingen
Anti-Sox2	AlexaFluor® 488	245610	IgG2a	1:20	BD Pharmingen
Anti-βIII Tubulin	AlexaFluor® 488	TUJ1	IgG2a	1:20	BD Pharmingen
CD10	FITC	HI10a	IgG1, k	1:5	BD Pharmingen
CD105	PE	266	IgG1, k	1:20	BD Pharmingen
CD117	PE	104D2	IgG1, k	1:5	BD Pharmingen
CD13	PE	L138	IgG1, k	1:5	BD Pharmingen
CD133	PE	AC133	IgG1	1:100	Miltenyi Biotec
CD14	FITC	MφP9	IgG2b, k	1:5	BD Pharmingen
CD140a (PDGFRα)	PE	αR1	IgG2a, k	1:5	BD Pharmingen
CD15 (SSEA-1)	APC	VIMC6	IgM	1:10	Miltenyi Biotec
CD166	PE	3A6	IgG1, k	1:5	BD Pharmingen
CD184 (CXCR4)	PE	12G5	IgG2a, k	1:5	BD Pharmingen
CD271 (LNGFR)	FITC	ME20.4-1.H4	IgG1	1:10	Miltenyi Biotec
CD29	PE	MAR4	IgG1, k	1:5	BD Pharmingen
CD31	FITC	WM59	IgG1, k	1:5	BD Pharmingen
CD34	APC	581	IgG1, k	1:50	BD Pharmingen
CD44	PE	515	IgG1, k	1:5	BD Pharmingen
CD45	FITC	5B1	IgG2a	1:10	Miltenyi Biotec
CD73	PE	AD2	IgG1, k	1:5	BD Pharmingen
CD90	FITC	5E+10	IgG1, k	1:100	BD Pharmingen
HLA-A,-B,-C	PE	G46-2.6	IgG1, k	1:5	BD Pharmingen
HLA-DR, DP, DQ	FITC	Tu39	IgG2a, k	1:5	BD Pharmingen
IgG1, k Isotype Control	FITC	MOPC-21	IgG1, k	1:5	BD Pharmingen
IgG1, k Isotype Control	PE	MOPC-21	IgG1, k	1:5	BD Pharmingen
IgG2a, k Isotype Control	FITC	G155-178	IgG2a, k	1:5	BD Pharmingen
IgG2b, k Isotype Control	FITC	27-35	IgG2b, k	1:5	BD Pharmingen
SSEA-4	FITC	MC813-70	IgG3	1:5	BD Pharmingen
SSEA-4	PE	MC813-70	IgG3	1:5	BD Pharmingen
Vimentin	PE	RV202	IgG1	1:20	BD Pharmingen

Supplemental Table 3. Primers used for RT-qPCR

Gene	**Primers (5'-3')**
Eag1	For TGGATTTTGCAAGCTGTCTG Rev GAGTCTTTGGTGCCTCTTGC
GAPDH	For TGATGACATCAAGAAGGTGGTGAAG Rev TCCTTGGAGGCCATGTGGGCCAT
GFAP	For GAAGCTCCAGGATGAAACCA Rev ACCTCCTCCTCGTGGATCTT
NSE	For CTGATGCTGGAGTTGGATGG Rev CCATTGATCACGTTGAAGGC
p0	For CCAGCTTTGCCCCTGTGGGT Rev AGTGTGCACGACGCTGAGCC
SOX2	For CATGTCCCAGCACTACCAGA Rev GTCATTTGCTGTGGGTGATG
CD133 (prominin)	For CAGAGTACAACGCCAAACCA Rev AAATCACGATGAGGGTCAGC
Oct4	For GTACTCCTCGGTCCCTTTCC Rev CAAAAACCCTGGCACAAACT