Supplementary material

Gene expression and functional comparison between multipotential stromal cells from lateral and medial condyles of knee osteoarthritis patients

Clara Sanjurjo-Rodriguez[1,2], Thomas G. Baboolal[1,3], Agata N. Burska[1,], Frederique Ponchel[1,], Jehan J. El-Jawhari[1,4], Hemant Pandit[1,3,5], Dennis McGonagle[1,3,5], Elena Jones[1,]*.

Supplementary materials and methods

Angiogenesis tube formation assay

To compare the paracrine angiogenic potential of MSCs from medial and lateral condyles, Matrigel-based angiotube formation assay was performed as previously described[1]. Briefly, MSCs were first seeded into 6 well plates containing StemMACS media and upon reaching 80% confluence, transferred into DMEM media supplemented with 1% FBS. Culture supernatants were collected after 72 hours, centrifuged at 300 x g for 10 minutes to eliminate cell debris and frozen at -80ºC until use. For the angiogenesis tube assay, 96-well culture plates (Corning) were pre-coated with 50 µl Matrigel (Corning Inc.), on top of which 10^4 human umbilical vein endothelial cells (HUVECs; Lonza, Switzerland) were seeded with the addition of MSC culture supernatants. Basal DMEM/1% FBS medium was used as negative control, and HUVEC medium (Endothelial Cell Growth Medium 2; PromoCell, Germany) was used as positive control. All conditions were assessed in duplicate. Plates were placed in the incubator at 37ºC and images were taken after three

hours. Tube lengths was measured using the Angiogenic analyzer plug-in for ImageJ, as described before[2].

Tables

Supplementary Table 1: Genes differentially expressed between multipotential stromal cells (MSC) and chondrocytes (CH).

Transcript	MSC/CH median fold-difference	p-value
SP7	CH BD	NA
HGF	CH LD	NA
ARNTL	CH LD	NA
IBSP	1020.3	<0.0001
IGF2	103.8	<0.0001
CXCL12	60.3	<0.0001
RUNX2	53.3	<0.0001
TNFSF11	29.7	<0.0001
MMP9	19.6	<0.0001
COL1A1	13.3	<0.0001
MMP13	11.8	0.0012
STMN2	10.1	0.0017
TIMP3	8.0	<0.0001
TGFB1	6.9	<0.0001
LEPR	6.4	<0.0001
SPHK1	6.3	<0.0001
S1PR1	5.8	<0.0001
NOTCH1	4.8	<0.0001
MMP2	4.1	<0.0001
ADAMTS4	4.1	<0.0001
PPARd	3.9	<0.0001
COL1A2	3.8	<0.0001
TGFBR1	3.7	<0.0001
SFRP1	3.6	0.0071
WISP1	3.2	<0.0001
ANKH	3.0	<0.0001
IGF1R	2.7	<0.0001
SPARC	2.5	0.0021
POSTN	2.4	0.0005
GREM1	2.1	0.0450

Transcript	CH/MSC median fold-difference	p-value
COMP	MSC BD	NA
CCL20	MSC BD	NA
NOS2	MSC BD	NA
CCR7	MSC BD	NA
LCN2	MSC LD	NA
IGF1	MSC LD	NA
IL10	MSC LD	NA
MMP3	105.2	<0.0001
FABP4	25.7	<0.0001
MMP1	21.4	<0.0001
CCL5	17.1	0.0004
DIO2	14.2	<0.0001
CCR1	12.6	0.0016
IGFBP3	4.4	<0.0001
ADAMTS5	3.4	<0.0001
CCL2	3.1	<0.0001
NGF	2.3	0.0364
PTH1R	2.1	0.0048

BD, below detection (in all donors); LD, low detection (detected in <50% donors); NA, not applicable. Fold-difference is calculated based on the median values for each group, Mann-Whitney test used for groups' comparisons.

Supplementary Table 2: Genes differentially expressed between $CD271^{+}CD45^{-}$ multipotential stromal cells (MSC) and $CD271^{-}CD45^{+}$ haematopoietic lineage cells (HLC).

Transcript	HLC/MSC median fold-difference	p-value
CCR5	BD in MSC	NA
CCR2	BD in MSC	NA
CCR6	BD in MSC	NA
DIRAS2	BD in MSC	NA
CCR1	LD in MSC	NA
IL1B	1346.9	<0.0001
MMP9	662.7	<0.0001
IL10	389.9	<0.0001
CCL5	212.6	<0.0001
CXCR4	135.6	<0.0001
CCR7	49.6	<0.0001
TNFa	7.9	0.0043
TGFB1	4.8	<0.0001
CCL20	3.5	0.0015
TIMP1	2.2	<0.0001

Transcript	MSC/HLC median fold-difference	p-value
SP7	BD in HLC	NA
SFRP1	BD in HLC	NA
STMN2	BD in HLC	NA
LEPR	LD in HLC	NA
NGF	LD in HLC	NA
WISP1	LD in HLC	NA
THBS4	LD in HLC	NA
MMP13	LD in HLC	NA
ASPN	LD in HLC	NA
ROR2	LD in HLC	NA
PTH1R	468.4	<0.0001
SFRP4	359.8	<0.0001
IGF2	326.8	<0.0001
DIO2	194.9	<0.0001
SOX9	166.0	<0.0001
ADAMTS5	137.4	<0.0001
CTGF	119.2	<0.0001
CYR61	101.7	<0.0001
VEGFC	100.1	<0.0001
NGFR	99.3	<0.0001
HGF	95.3	<0.0001
CXCL12	94.4	<0.0001
DDR2	86.3	<0.0001
IBSP	70.7	<0.0001
MMP3	66.2	<0.0001
IGFBP3	63.4	<0.0001
MMP2	50.3	<0.0001
ADAMTS4	40.4	<0.0001
COL1A2	39.1	<0.0001
POSTN	37.1	0.0004
TIMP3	36.8	<0.0001
TGFB2	35.2	<0.0001
TNFSF11	31.5	<0.0001
COL1A1	31.5	<0.0001
TGFB3	24.3	<0.0001
SERPINE1	13.9	<0.0001
IL6	13.7	<0.0001
SPARC	12.7	<0.0001
CCL2	10.2	<0.0001
PPARg	10.2	<0.0001
PTHLH	8.2	0.0003
TIMP2	4.7	<0.0001
SPP1	4.0	0.0039
PTGS2	3.6	<0.0001
IGF1R	3.6	<0.0001
RUNX2	3.3	<0.0001
ANKH	3.2	<0.0001
MMP14	3.0	<0.0001
TGFBR1	2.9	0.0129
ACAN	2.8	0.0400
MMP1	2.7	0.0129
VEGFA	2.5	<0.0001
S1PR1	2.2	0.0015
FABP4	2.2	0.0301
SPHK1	2.1	0.0107

BD, below detection (in all donors); LD, low detection (detected in <50% donors). Fold-difference is calculated based on the median values for each group, Mann-Whitney test used for groups' comparisons.

Supplementary table 3: Taqman probes used to assess the gene expression.

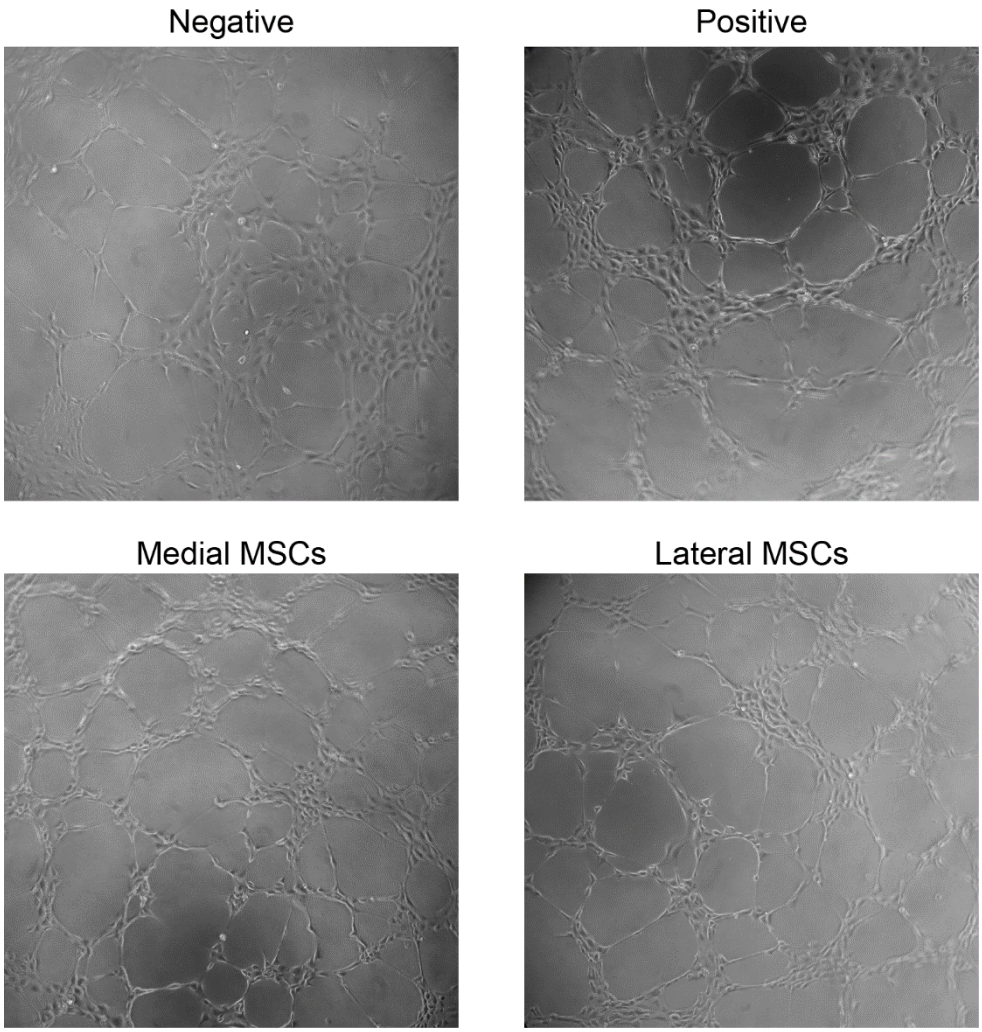
Gene symbol	Gene name	Assay number	Bibliography
ACAN	aggrecan	HS00153936-m1	[3, 4, 5, 6, 7]
ADAMTS4	a disintegrin and metalloproteinase with thrombospondin motifs 4	HS00192708-m1	[3, 4, 6, 8, 9]
ADAMTS5	a disintegrin and metalloproteinase with thrombospondin motifs 5	HS01095524-m1	[3, 4, 6, 8, 10, 9, 11]
ANKH	human homolog of the murine progressive ankylosis gene	HS01064613-m1	[3]
ARNTL	aryl hydrocarbon receptor nuclear translocator-like protein 1	HS00154147-m1	[12]
ASPN	asporin	HS01558901-m1	[13, 6]
BCL2	BCL2, apoptosis regulator	HS00608023-m1	[14, 15]
BGLAP	Bone Gamma-Carboxyglutamate Protein	HS01587814-g1	[3,16]
BMPR1B	Bone Morphogenetic Protein Receptor Type 1B	HS01010965-m1	[17]
CCL19	C-C Motif Chemokine Ligand 19	HS00171149-m1	[8]
CCL2	C-C Motif Chemokine Ligand 2	HS00234140-m1	[13, 6, 18, 19]
CCL20	C-C Motif Chemokine Ligand 20	HS01011368-m1	[6]
CCL5	C-C Motif Chemokine Ligand 5	HS00982282-m1	[8, 19]
CCR1	C-C Motif Chemokine Receptor 1	HS00174298-m1	[20]
CCR10	C-C Motif Chemokine Receptor 10	HS00706455-s1	[20]
CCR2	C-C Motif Chemokine Receptor 2	HS00356601-m1	[6, 19]
CCR3	C-C Motif Chemokine Receptor 3	HS99999027-s1	[20]
CCR5	C-C motif chemokine receptor 5	HS00152917-m1	[19]
CCR6	C-C Motif Chemokine Receptor 6	HS00171121-m1	[6]
CCR7	C-C Motif Chemokine Receptor 7	HS04398702-m1	[20]
COL10A1	Collagen Type X Alpha 1 Chain	HS00166657-m1	[4, 5, 6, 9, 17]
COL1A1	Collagen Type I Alpha 1 Chain	HS01076777-m1	[3, 7]
COL1A2	Collagen Type I Alpha 2 Chain	HS01028971-m1	[3, 6, 7]
COL2A1	Collagen Type II Alpha 1 Chain	HS00264051-m1	[3, 4, 5, 10, 21]
COMP	Cartilage Oligomeric Matrix Protein	HS00164359-m1	[3, 21, 7]
CTGF	Connective Tissue Growth Factor	HS00170014-m1	[6]
CXCL12	C-X-C Motif Chemokine Ligand 12	HS00171022-m1	[22, 6, 7]
CXCR1	C-X-C Motif Chemokine Receptor 1	HS01921207-m1	[6]
CXCR4	C-X-C Motif Chemokine Receptor 4	HS00607978_S1	[6]
CYR61	Cysteine Rich Angiogenic Inducer 61	HS00155479-m1	[6]
DDR2	iscoidin Domain Receptor Tyrosine Kinase 2	HS01025956-m1	[4]
DIO2	Iodothyronine Deiodinase 2	HS00255341-m1	[13, 8]

DIRAS2	DIRAS Family GTPase 2	HS01107862-m1	[23]
FABP4	Fatty Acid Binding Protein 4	HS00609791-m1	[6]
GDF5	Growth Differentiation Factor 5	HS00167060-m1	[3, 6, 8, 18, 17, 7]
GDF6	Growth Differentiation Factor 6	HS01377663-m1	[3, 22]
GREM1	Gremlin 1, DAN Family BMP Antagonist	HS00171951-m1	[18]
HGF	Hepatocyte Growth Factor	HS00300159-m1	[3]
HPRT1	Hypoxanthine Phosphoribosyltransferase 1	HS99999909-m1	House-keeping gene
IBSP	integrin binding sialoprotein	HS00173720-m1	[3]
IGF1	Insulin Like Growth Factor 1	HS03986524-m1	[3, 22, 5, 10, 24, 25]
IGF1R	Insulin Like Growth Factor 1 Receptor	HS00609566-m1	[24]
IGF2	Insulin Like Growth Factor 2	HS00171254-m1	[3, 22, 5, 26]
IGFBP3	Insulin Like Growth Factor Binding Protein 3	HS00426289-m1	[27, 7]
IL10	Interleukin 10	HS00961622-m1	[28]
IL1B	Interleukin 1 Beta	HS01555413-m1	[3, 4, 5, 8, 28, 9, 11]
IL6	Interleukin 6	HS00174131-m1	[3, 4, 5, 5, 8, 28, 9, 11]
LCN2	Lipocalin 2	HS01008571-m1	[25]
LEPR	Leptin Receptor	HS00174492-m1	[9]
MMP1	Matrix Metallopeptidase 1	HS00899658-m1	[3, 4, 5, 6, 8, 9]
MMP13	Matrix Metallopeptidase 13	HS00942589-m1	[23, 3, 4, 5, 6, 8, 10, 9, 11]
MMP14	Matrix Metallopeptidase 14	HS00237119-m1	[21]
MMP2	Matrix Metallopeptidase 2	HS01548728-m1	[3, 4, 5, 6, 21]
MMP3	Matrix Metallopeptidase 3	HS00968308-m1	[3, 4, 22, 5, 6, 8, 9]
MMP9	Matrix Metallopeptidase 9	HS00957562-m1	[3, 4, 5, 25, 9, 11]
NGF	Nerve Growth Factor	HS00171458-m1	[6, 18]
NGFR	Nerve Growth Factor Receptor	HS00182120-m1	[6, 18]
NOS2	Nitric Oxide Synthase 2	HS01075529-m1	[3, 8, 9, 11]
NOTCH1	Notch 1	HS01062014_m1	[11]
POSTN	Periostin	HS01566750-m1	[13,22]
PPARd	Peroxisome Proliferator Activated Receptor Delta	HS00602622-m1	[29]
PPARg	Peroxisome Proliferator Activated Receptor Gamma	HS01115513-M1	[22, 6, 7]
PSIP1	PC4 And SFRS1 Interacting Protein 1	HS01045714-g1	[23]
PTGS2	Prostaglandin-Endoperoxide Synthase 2	HS00153133-m1	[30]
PTH1R	Parathyroid Hormone 1 Receptor	HS00896824-m1	[15, 10]
PTHLH	Parathyroid Hormone Like Hormone	HS00174969-m1	[5, 15, 10, 17]
ROR2	Receptor Tyrosine Kinase Like Orphan Receptor 2	HS00171695-m1	[31]
RUNX2	Runt Related Transcription Factor 2	HS00234692-m1	[3, 4, 6, 10, 17]

S1PR1	Sphingosine-1-Phosphate Receptor 1	HS00173499-m1	[3]
SERPINE1	Serpin Family E Member 1	HS00167155-m1	[26]
SFRP1	Secreted Frizzled Related Protein 1	HS00610060-m1	[32]
SFRP4	Secreted Frizzled Related Protein 4	HS00180066-m1	[7]
SOX9	SRY-Box 9	HS00165814-m1	[4, 6, 17, 16]
SP7	Sp7 Transcription Factor	HS00541729-m1	[16]
SPARC	Secreted Protein Acidic And Cysteine Rich	HS00277762-m1	[6, 7]
SPHK1	Sphingosine Kinase 1	HS00184211-m1	[3]
SPP1	Secreted Phosphoprotein 1	HS00959010-m1	[3, 4]
STMN2	Stathmin 2	HS00975800-m1	[23, 18]
TGFB1	transforming growth factor beta 1	HS00998133-m1	[3, 4, 22, 5, 6, 10, 25]
TGFB2	transforming growth factor beta 2	HS00234244-m1	[3, 4, 22, 5]
TGFB3	Transforming Growth Factor Beta 3	HS01085997-m1	[3, 4, 5, 21]
TGFBR1	Transforming Growth Factor Beta Receptor 1	HS00610319-m1	[6, 10]
TGFBR2	transforming growth factor beta receptor 2	HS00559661-m1	[6, 10]
TGFBR3	Transforming Growth Factor Beta Receptor 3	HS00234257-m1	[10]
THBS4	Thrombospondin 4	HS00170261-m1	[23]
TIMP1	TIMP metallopeptidase inhibitor 1	HS00171558-m1	[6, 21]
TIMP2	TIMP metallopeptidase inhibitor 2	HS01091319-m1	[6, 26]
TIMP3	TIMP Metallopeptidase Inhibitor 3	HS00927214-m1	[6, 21]
TNFa	Tumor Necrosis Factor	HS99999043-m1	[3, 13,22, 5, 8, 28, 11]
TNFRSF11B	TNF Receptor Superfamily Member 11b	HS00900360-m1	[13, 5, 6, 10]
TNFSF11	TNF Superfamily Member 11	HS01092186-m1	[3, 4, 13,22, 5, 6, 10, 32]
VEGFA	Vascular Endothelial Growth Factor A	HS00900058-m1	[3, 4, 5, 6, 10, 11]
VEGFC	Vascular Endothelial Growth Factor C	HS01099206-m1	[3, 5, 7]
WISP1	WNT1 Inducible Signaling Pathway Protein 1	HS04234730-m1	[3, 13]
WNT10b	Wnt Family Member 10B	HS00559664-m1	[13]

Figures

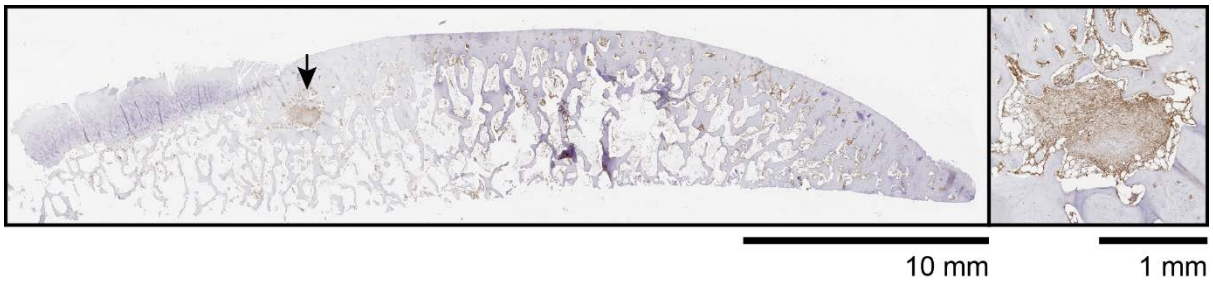
Supplementary Figure 1



Supplementary figure 1: Representative images illustrating angiogenic tube formation assay in Matrigel. Negative: human umbilical vein endothelial cells (HUVECs) seeded in DMEM supplemented with 1% FCS; Positive: HUVECs seeded in HUVEC medium (Endothelial Cell Growth Medium 2), more tubes and junctions are evident; Medial MSCs: HUVECs seeded in conditioned-medium DMEM 1% FCS obtained from medial condyle MSCs; Lateral MSCs: HUVECs seeded in conditioned-medium DMEM 1% FCS obtained from lateral condyle MSCs. No notable differences

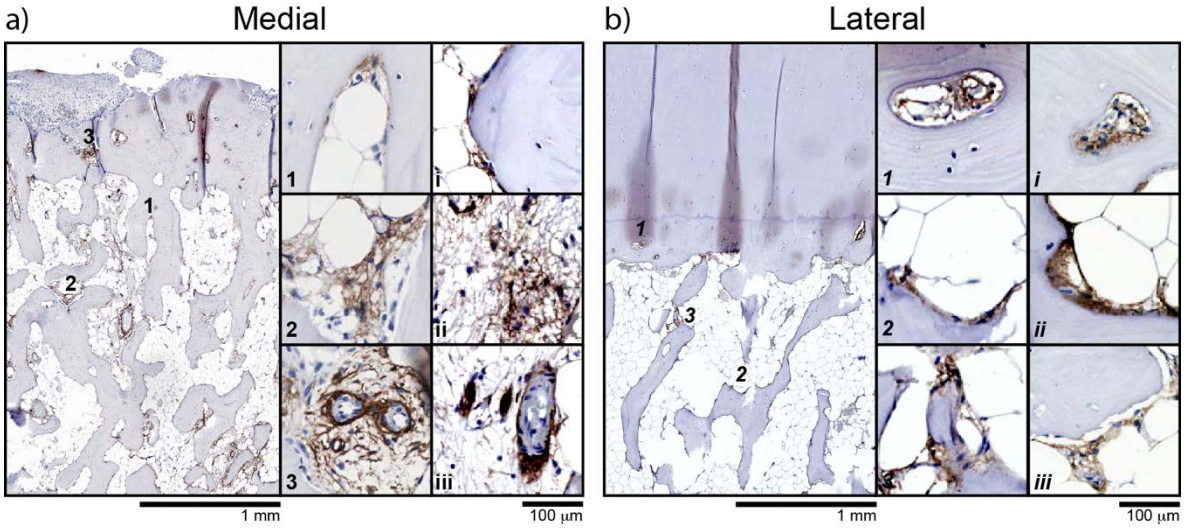
in the number of tubes and junctions are evident between media conditioned by medial and lateral condyle MSCs.

Supplementary Figure 2



Supplementary figure 2: Immunohistochemistry showing CD271 positivity in stromal tissue of a cyst (arrow and magnified image) within the medial condyle of OA donor tissue.

Supplementary Figure 3



Supplementary Figure 3: Immunohistochemistry staining showing CD271 positivity within medial (a) and lateral (b) tibial plateau sections. a) Medial tibial plateau sections show region of cartilage denudation with CD271 positivity on bone lining

cells (1 and i), positivity in stromal tissue (2 and ii) and around blood vessels (3 and iii). Magnified images show corresponding (1-3) areas and similar features from other donors shown in (i-iii). b) Lateral tibial plateau images showing areas of CD271 positivity in the presence of a full thick cartilage layer. Positivity was seen in areas of vascular channel invasion (*1* and *i*) and characteristic bone lining MSCs (*2-3* and *ii-iii*). Magnified images show corresponding *(1-3)* areas and similar features from other donors shown in *(i-iii)*.

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