Nucleus pulposus phenotypic markers to determine stem cell differentiation: fact or fiction?

Supplementary Material

Supplementary Table I: IVD samples processed at Sheffield Hallam University: Patient Details.

Reference		Source	IVD Level	Infiltrating Cells	Average Grade	Classification	DE	IHC
HD	1	Surgical	L4/L5	No	3.0	I	✓	✓
HD	2	Surgical	L5/S1	Yes	7.0	I	✓	
HD	3	Surgical	L4/L5	No	4.0	MD	✓	
HD	5	Surgical	L5/S1	No	8.5	SD	✓	
HD	9	Surgical	L5/S1	No	2.0	ND		✓
HD	17	Surgical	L5/S1	Yes	4.0	ND	✓	
HD	21	Surgical	L4/L5	Yes	4.0	I	✓	
HD	24	Surgical	L5/S1	No	2.0			✓
HD	25	Surgical	L4/L5	No	2.0	I	✓	
HD	26	Surgical	L5/S1	No	5.0	I	✓	
HD	30	PM (SHU)	L4/L5	No	2.0		✓	
HD	31	PM (SHU)	L3/L4	No	3.0	ND		✓
HD	34	Surgical	L5/S1	Yes	12.0	SD	✓	
HD	44	Surgical	L5/S1	No	2.0	ND	✓	
HD	45	Surgical	L5/S1	No	8.0	SD	✓	
HD	53	Surgical	L5/S1	No	7.0	MD	✓	
HD	54	Surgical	L4/L5	No	6.0	SD	✓	✓
HD	56	Surgical	L5/S1	Yes	5.0	MD	✓	✓
HD	57	Surgical	L5/S1	No	6.0	MD	✓	✓
HD	58	Surgical	L5/S1	No	3.5	SD	✓	
HD	59	Surgical	L5/S1	No	6.0	SD	✓	
HD	61	Surgical	L5/S1	No	7.5	MD	✓	✓
HD	63	Surgical	L5/S1	No	9.0	SD	✓	✓
HD	65	Surgical	L4/L5	No	10.0	SD	✓	
HD	66	Surgical	L3/L4	Yes	5.0	I	✓	
HD	71	Surgical	L5/S1	Yes	3	MD		✓
HD	75	Surgical	L3/L4	No	8.0	MD	✓	✓
HD	79	Surgical	L4/L5	No	3.0	ND	✓	✓
HD	85	Surgical	L2/L3	No	8.0	I	✓	
HD	86	Surgical	L5/S1	Yes	1.0	I	✓	
HD	92	Surgical	L5/S1	No	3.5	ND	✓	
HD	93	Surgical	L5/S1	Yes	6.0	MD	✓	✓
HD	94	Surgical	L5/S1	No	6.0	MD	✓	
HD	97	Surgical	L5/S1	No	6.0	MD	✓	✓

HD	98	Surgical	L3/L4	No	6.0	MD	✓	
HD	109	Surgical	L4/L5		6.0			
HD	133	Surgical	L4/L5	No	3.5			✓
HD	144	Surgical	L5/S1	No	5.0		✓	✓
HD	145	Surgical	L4/L5	No	11	SD	✓	
HD	146	Surgical	L5/S1	No	5.85	MD	✓	
HD	148	Surgical	C5/C6	No	6.0	MD	✓	
HD	151	Surgical	L5/S1	No	5.3	MD	✓	
HD	153	Surgical	L5/S1	Yes	5.0	MD	✓	
HD	154	Surgical	L4/L5	No	3.0	MD	✓	
HD	156	Surgical	L4/L5	Yes	3.0	ND	✓	
HD	157	Surgical	L5/S1	Yes	5.0	I	✓	
HD	158	Surgical	L4/L5	No	3.0	I	✓	✓
HD	159	Surgical	L4/L5	Yes	9.0	I	✓	
HD	160	Surgical	C3/C5	No	6.0	MD	✓	
HD	166	Surgical	L4/L5	No	8.0	SD	✓	
HD	170	Surgical	L5/S1	No	8.0	SD		✓
HD	174	Surgical	L5/S1	No	7.0	MD	✓	
HD	175	Surgical	L5/S1	No	7.0	SD	✓	
HD	177	Surgical	L4/L5	No	2.0	N	✓	
HD	184	Surgical	L4/L5	Yes	5.0	SD	✓	
HD	192	Surgical	L5/S1	No	8.0	SD	✓	
HD	194	Surgical	L4/L5	No	11.0	SD	✓	✓
HD	195	Surgical	C6/C7	No	9.0	SD	✓	
HD	197	Surgical	L4/L5	No	8.0	SD	✓	
HD	203	Surgical	S1	No	3.5			✓
HD	207	Surgical	L5/S1	No	8.0	MD	✓	✓
HD	219	Surgical	L5/S1	No	10.5	I	✓	
HD	225	Surgical	L5/S1	No	10.0	SD	✓	✓
HD	228	Surgical		No	6.0	I	✓	
HD	229	Surgical	L5/S1	No	6.0	MD	✓	✓
HD	231	Surgical	L4/L5	No	5.0	MD	✓	✓
HD	232	Surgical	L5/S1	No	8.0	SD	✓	✓
HD	233	Surgical	L5/S1	No	9.5	SD	✓	✓
HD	234	Surgical	L5/S1	No	8.5	SD	✓	✓
HD	243	Surgical	L4/L5	Yes	6.0	I	✓	
HD	246	Surgical	L5/S1	Yes	6.0	I	✓	
HD	253	Surgical	C5/C6	Yes	4.0	I	✓	
HD	254	Surgical	L4/L5	Yes	6.0	I	✓	✓
HD	257	Surgical	L5/S1	Yes	6.0	I	✓	
HD	266	Surgical	L5/S1	No	4.0		✓	✓
HD	276	Surgical	C5/C6	No	6.0	MD	✓	✓
HD	281	Surgical	L4/L5	No	3.0	ND	✓	

Supplementary Table II: IVD samples processed at Utrecht: Patient Details

Reference		Source	IVD Level	Thompson Grade	DE	IHC
HD	S06 115	PM (UT)	L4/5	1		✓
HD	S08 263	PM (UT)	L4/5	1		✓
HD	S06 69	PM (UT)	L4/5	1		✓
HD	S06 123	PM (UT)	L4/5	1		✓
HD	S07 099	PM (UT)	L4/5	2		✓
HD	S07184	PM (UT)	L4/5	2		✓
HD	S06116	PM (UT)	L4/5	2		✓
HD	S06224	PM (UT)	L4/5	2		✓
HD	S07 099	PM (UT)	L4/5	2		✓
HD	S07203	PM (UT)	L4/5	3		✓
HD	S07229	PM (UT)	L4/5	3		✓
HD	S06127	PM (UT)	L4/5	3		✓
HD	S07098	PM (UT)	L4/5	3		✓
HD	S07137	PM (UT)	L4/5	3		✓
HD	S07209	PM (UT)	L4/5	4		✓
HD	S07250	PM (UT)	L4/5	4		✓
HD	W07243	PM (UT)	L4/5	4		✓
HD	S06141	PM (UT)	L4/5	4		✓
HD	S07256	PM (UT)	L4/5	4		✓
HD	S06150	PM (UT)	L4/5	5		✓
HD	S06251	PM (UT)	L4/5	5		✓
HD	S06287	PM (UT)	L4/5	5		✓
HD	S0741	PM (UT)	L4/5	5		✓
HD	S06165	PM (UT)	L4/5	5		✓

Supplementary Table III: Articular Cartilage samples: Patient Details.

Reference		Source	Anatomical Compartment	Macroscopic Grade	Microscopic Grade	DE	IHC
HC	4 (3)	Surgical	Medial femoral condyle	1	4	✓	✓
HC	5 (2)	Surgical	Posterior femoral condyle	0	5	✓	✓
HC	7 (1)	Surgical	Posterior femoral condyle	0	3.5	✓	✓
HC	9 (1)	Surgical	Posterior femoral condyle	0	2.5	✓	✓
HC	10 (1)	Surgical	Trochlear	0	5	✓	✓
HC	11 (1)	Surgical	Posterior condyle	0	3.5	✓	✓
HC	16 (1)	Surgical	Posterior femoral condyle	1	2.5	✓	✓
HC	17 (1)	Surgical	Medial femoral condyle	0	3	✓	✓
HC	18 (1)	Surgical	Lateral posterior femoral condyle	1	3	✓	✓
HC	19 (1)	Surgical	Lateral femoral condyle	0	5	✓	✓

Table IV: Predesigned primer probes for qRT-PCR

Gene	Gene Symbol	Catalogue no.
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	Hs02758991_g1
18S Ribosomal RNA	18S	Hs03003631_g1
Forkhead Box F1	FOXF1	Hs00230962_m1
Paired Box 1	PAX1	Hs01071292_m1
Keratin 19	KRT19	Hs01051611_gH
Keratin 18	KRT18	Hs02827483_g1
Cluster of differentiation 24	CD24	Hs03044178_g1
Glucose transporter 1	SLC2A1	Hs00892681_m1
Brachyury	T	Hs00610080_m1
carbonic anhydrase XII	CA12	Hs01080909_m1
Sonic Hedgehog	SHH	Hs00179843_m1
Laminin Alpha 5	LAMA5	Hs00966585_m1
Laminin gamma 2	LAMC2	Hs01043711_m1
Ovostatin 2	OVO2	Hs00416380_m1
Integrin Binding Sialoprotein	IBSP	Hs00173720_m1

SupplementaryTable V: Target antibodies used for IHC

Target Antibody	Clonality	Optimal Dilution	Antigen Retrieval Method	Secondary Antibody	Serum Block
FOXF1 (ab23194)	Rabbit Polyclonal	1:100	Heat	Goat anti-rabbit	Goat
PAX1 (ab203065)	Rabbit Polyclonal	1:400	Enzyme	Goat anti-rabbit	Goat
KRT-19 (ab7754)	Mouse monoclonal	1:400	None	Rabbit anti-mouse	Rabbit
LAM-5 (ab78286)	Mouse monoclonal	1:800	Enzyme	Rabbit anti-mouse	Rabbit
HIF1α (ab16066)	Mouse monoclonal	1:100	None	Rabbit anti-mouse	Rabbit

Supplementary Table V: Target antibodies used for IHC, their optimal concentrations and antigen retrieval methods. Heat antigen retrieval consisted of 10 minute microwave irradiation in 0.05M Tris buffer, pH 9.5 pre-heated to 60˚C. Enzyme antigen retrieval consisted of 30-minute incubation in TBS; 20 mM tris, 150 mM sodium chloride, 46.8mM calcium chloride dihydrate pH 7.5, containing 0.01% w/v α-chymotrypsin from bovine pancreas at 37°C.