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Differential activation of NOTCH1 pathway in HNSCC cell lines of different anatomical sites

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Supplementary Data

Suppl. Table S1A — Primers for m-RNA expression analysis of the genes and copy number variation analysis of the genes with details of antibodies used in the study

Gene	Sequence	Product size
NOTCH1	5'-AGGACCTCATCAACTCACACGC-3' 5'-CGTTCTTCAGGAGCACAACTGC-3'	117
JAG1	5'-TGTCTGTGTCCCACTGGTTTCTC-3' 5'-AGTTCTTGCCCTCATAGTCCTCG-3'	148
JAG2	5'-CTCTCTGTGAGGTGGATGTCGACC-3' 5'-ACGGAGCAGTTCTTGCCACCAA-3'	127
HES1	5'-CCAAGCTGGAGAAGGCGGACATTC-3' 5'-ACGTGGACAGGAAGCGGGTCAC-3'	165
HEY1	5'-CGAGGTGGAGAAGGAGAGTG-3' 5'-CTGGGTACCAGCCTTCTCAG-3'	177
CD44	5'GGACAAGTTTTGGTGGCACT-3 5'-TCCGTCCGAGAGATGCTGTA-3'	141
FBXW7	5'-GTGGACCAGAGAAATTGCTTGC-3' 5'-GGGTTCCAGGAATGAAAGCACA-3'	161
HIF1α	5'-TTAGAACCAAATCCAGAGTCAC-3' 5'-TATTCACTGGGACTATTAGGCT-3'	125
VEGF	5'-GAGATGAGCTTCCTACAGCAC-3' 5'-TCACCGCCTCGGCTTGTCACAT-3'	345

Suppl. Table S1B — Antibody details

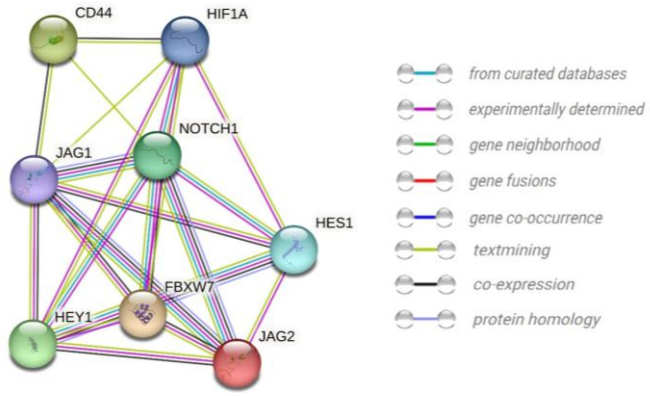
Primary Antibody	Catalog #
NICD	ab8925
HES1	ab71559
CD44 (HCAM)	Sc7297
FBXW7	ab109617
HIF1α	sc10790
VEGF	sc7269
α-tubulin	Sc5286
Secondary Antibody	
goat anti-rabbit	sc-2004
goat anti-mouse IgG	sc-2005

Suppl. Table S2A — Details of gel percentage in the Western blot analysis of each protein according to their molecular weight

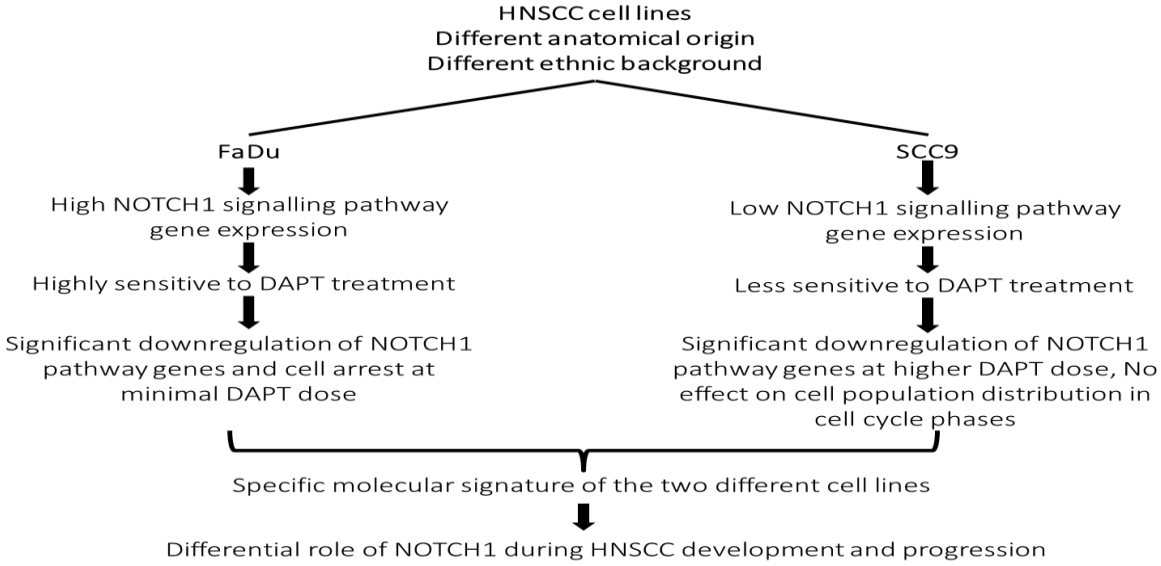
Protein name	Molecular weight (kDa)	Gel percentage
NICD (NOTCH1 intracellular domain)	105	8%
HES1	20	12%
HIF1α	135	8%
FBXW7	69	12%
CD44	92	10%
VEGF	21	12%

Suppl. Table S2A — Details of the band read of each protein at different DAPT concentration, control and DMSO treatment by image J software in triplicate

		DMSO			IC_{30}			IC_{50}			IC_{70}		
FaDu	VEGF	1.01	1.01	0.98	0.99	0.95	0.91	0.84	0.83	0.83	0.72	0.71	0.70
	HIF1α	0.98	0.96	0.98	0.99	0.98	0.99	0.79	0.75	0.79	0.65	0.65	0.67
	FBXW7	1.02	0.99	0.97	1.01	0.95	0.93	1.00	0.96	0.94	0.99	0.97	0.95
	CD44	1.00	0.98	0.97	1.00	0.99	0.99	0.75	0.72	0.74	0.64	0.63	0.65
	HES1	0.99	0.98	0.99	0.86	0.85	0.85	0.75	0.74	0.77	0.60	0.58	0.62
	NICD	0.97	0.99	0.98	0.86	0.88	0.84	0.77	0.79	0.77	0.62	0.61	0.63
SCC9	VEGF	0.98	1.02	1.07	1.00	0.97	0.99	0.97	0.97	1.00	0.73	0.73	0.75
	HIF1α	0.97	1.02	1.02	0.97	0.99	0.98	0.99	0.97	0.99	0.79	0.77	0.77
	FBXW7	1.02	1.02	1.09	0.97	1.04	0.94	0.97	1.01	0.96	0.99	0.94	1.00
	CD44	0.98	1.03	1.04	0.96	0.99	0.98	0.96	0.97	1.00	0.70	0.73	0.73
	HES1	0.96	0.97	1.03	0.95	0.98	0.97	0.94	0.97	0.99	0.78	0.77	0.80
	NICD	0.97	1.04	1.06	0.95	0.98	1.00	0.93	0.99	1.03	0.77	0.80	0.84



Suppl. Fig. S1 — Protein-protein interaction network determined by STRING (version: 11.5) analysis for NOTCH1 pathway associated genes with minimum required interaction score as 0.400 (medium confidence)



Suppl. Fig. S2 — Showing graphical conclusion of the study