

Supplementary Materials and Methods.

Immunohistochemistry Assay

Primary tumor tissue microarrays were stained for Hes-1 protein expression using a polyclonal antibody HES1: Millipore Ab5702 (Rabbit Polyclonal). Slides containing the sections were baked at 60°C for 1 h and then de-paraffinized in a series of xylene baths. The slides were then rehydrated in graded alcohol. To retrieve antigenicity, the tissue sections were heated using a microwave in 10 mM citrate buffer (pH 6.0) three times for 3 min each. The sections were then immersed in methanol containing 0.3% hydrogen peroxide for 20 min to block endogenous peroxidase activity and were incubated in 2.5% blocking serum to reduce the staining background. Sections were incubated overnight at 4°C using the polyclonal HES1 antibody at a dilution of 1:100. The sections were processed using the standard avidin-biotin system for staining according to the manufacturer's protocols (Vector Laboratories, Burlingame, CA). Diaminobenzidine was used as a chromogen, and hematoxylin was used for counterstaining. Slides were scored without knowing the clinical features and treatment outcome.

PCR Assay

The PCR reaction was performed in a 12.5- μ l volume containing 100ng of genomic DNA, 7% DMSO, 1.5 mM deoxynucleotide triphosphates, 6.7 mM MgCl₂, 16.6 mM (NH₄)₂SO₄, 67 mM Tris, 10 mM β -mercaptoethanol, 6.7 μ M EDTA, 0.8 μ M of both the forward and the reverse primer, and 0.625 unit of Hot star Taq DNA Polymerase (Qiagen, Inc., Chatsworth, CA). Amplification was carried out with an initial denaturing step at 95°C for 15 min, followed by 40 cycles of 95°C for 30s, 68°C for 1min, and 72°C for 1min in a Mastercycler (Eppendorf).

GFP primers:

Forward; pGF1F: 5'-tcttcaccgacaagatcatcc-3'

Reverse; pGF1R: 5'-gtccaccacgaagctgtagta-3'

Quantitative real-time reverse transcription-PCR

Primers obtained from Applied Biosystems.

Notch-1	hs00413187_m1	DLL4	hs00184092_m1
Notch-2	hs00225747_m1	Jagged1	hs00164982_m1
Notch-3	hs00166432_m1	Jagged2	hs00171432_m1
Notch-4	hs00270200_m1	ACTB	hs99999903_m1
HES1	hs00172878_m1	Nanog	Hs02387400_g1
HES5	hs01387464_g1	SOX2	Hs01053049_s1
HEY1	hs00232618_m1	OCT4	Hs00999632_g1
Hey2	hs00232622_m1	KLF4	Hs00358836_m1
DLL1	hs00194509_m1	Lin28A	Hs00702808_s1
DLL3	hs01085096_m1		