Supplementary File 4

Primer sequence overview

Primers were custom made with the software Perlprimer (http://perlprimer.sourceforge.net/) or obtained from the RT Primer Database (http://medgen.ugent.be/rtprimerdb/), and are listed below. For normalization the following reference genes were used: beta-2-microglobulin, glyceraldehyde 3-phosphate dehydrogenase, hypoxanthine phosphoribosyltransferase 1 and ribosomal protein L19. The nonparametric Mann-Whitney test was used for all statistical comparisons. A p-value of less than 0.05 was considered significant.

Gene Symbol	Name	Forward Primer	Position	Length	Tm	Reverse Primer	Position	Length	Tm	Amplicon Size
KRT19	keratin 19	CTTCCGAACCAAGTTTGAGAC	633	21	61.48	AGCGTACTGATTTCCTCCTC	815	20	61.36	182
KRT7	keratin 7	GGACATCGAGATCGCCACCT	1281	20	60	ACCGCCACTGCTACTGCCA	1386	19	60	124
PROM1	prominin 1	TGGATGCAGAACTTGACAACGT	1049	22	60	ATACCTGCTACGACAGTCGTGGT	1159	23	60	133
GSTP1	glutathione S-transferase pi 1	TGTCGGGTGGGTAAGGAGATAG	583	22	60	TTGCCCTTAGGAGACTCCAAAC	788	22	60	225
HNF4A	hepatocyte nuclear factor 4, alpha	GTACTCCTGCAGATTTAGCC	367	20	60.20	CTGTCCTCATAGCTTGACCT	528	20	61.01	161
ABCC2	ATP-binding cassette, sub-family C (CFTR/MRP), member 2	TACCACCAAACTCTATCTTGCTAAGC	318	26	60	AGTACAAGGGCCAGCTCTATGG	377	22	60	81
JAG1	jagged 1 (Alagille syndrome)	AATGGGTGGAAAGGAAAGAC	2495	20	60.20	TGTTTCGGGCTATGTTACAG	2648	20	60.05	153
JAG2	jagged 2	CTGGAGGGTGACTATTACTG	1949	20	58.88	AGTCGTCAATGTTCTCATGG	2207	20	60.05	258
NOTCH1	Notch homolog 1, translocation-associated (Drosophila)	GTGACTGCTCCCTCAACTTCAAT	4429	23	60	CTGTCACAGTGGCCGTCACT	4500	20	60	91
NOTCH2	Notch homolog 2 (Drosophila)	GTCTCAGTGGATATAAGTGTCTC	2465	23	59.78	ACCAGATTGTCACAAGTTCCT	2582	21	61.67	117
NOTCH3	Notch homolog 3 (Drosophila)	TCTTCCAGATTCTCATCCGA	5735	20	60.05	CATCCACAGCATTGACATCAG	5884	21	61.69	149
S100A6	S100 calcium binding protein A6	CTGCAGGATGCTGAAATTGC	455	20	60	GGAAGTTCACCTCCTGGTCCTT	503	22	60	70
CTBP2	C-terminal binding protein 2	CATCAGCGCCTTGGTCAGTA	1524	20	60	TGCCTGGCGGATATCTGTATG	1584	21	60	81
FAM57A	family with sequence similarity 57, member A	ACTCTTCGAAACTTCCTAAGTC	365	22	59.78	CTTTAGCTGAATCAGAACCCT	550	21	59.77	185
TACSTD2	tumor-associated calcium signal transducer 2	GTAGCCTCATTTACCATCGT	1794	20	59.55	TCCTCAAAGACATCCAAACTG	1895	21	60.26	101
MTSS1	metastasis suppressor 1	TAGTGTTTAAGAAAGCAAGCAAGTC	4156	25	60	GAGGGTTCGGTCAGAAATGTG	4206	21	60	71
ANXA3	annexin A3	GACATTAGTTCCGAAACATCTG	736	22	59.40	TTTCATCTCTTCTGCCATCTG	808	21	60.12	72
VASP	vasodilator-stimulated phosphoprotein	GAGAAGAACAGCACAACCT	1272	19	59.83	GAAGCTCCTGTTTCACCC	1395	18	60.23	123
RAB25	RAB25, member RAS oncogene family	GACCAATCTACTCTCCCGA	300	19	60.06	CACGATAGTACGCCGAG	475	17	59.19	175
RAB27B	RAB27B, member RAS oncogene family	GACCAATCTACTCTCCCGA	300	19	60.06	CACGATAGTACGCCGAG	475	17	59.19	175
PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	GGCATTCTTTGCAATACTGCTTAA	5518	24	60	CATCTGCCGATAGCACAGTGA	5586	21	60	89
LAMB1	laminin, beta 1	TTCCAAGTTGCCAGCCC	2916	17	60.28	GCCAAGCACCTTTCACAG	3033	18	59.40	117
LAMC2	laminin, gamma 2	GAAACACTAACATTCCTGCCTC	2526	22	59.75	TTCCGCTTCCGACTCCT	2726	17	60.37	200
IQGAP1	IQ motif containing GTPase activating protein 1	GAAAGCCCAGGAAATCCAG	1867	19	60.67	TCCATACAAGCCAACATCAG	2065	20	60.05	198
IQGAP2	IQ motif containing GTPase activating protein 2	AGTGGTTAAGAGCGATGGA	565	19	60.60	GATTTCCTCCTCTGTGAAGTC	758	21	60.12	193
B2M	beta-2-microglobulin	ACAGCCCAAGATAGTTAAGTG	383	21	59.77	ATCTTCAAACCTCCATGATGC	443	21	60.74	60
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	CAAGATCATCAGCAATGCCT	533	20	60.78	CAGGGATGATGTTCTGGAGAG	726	21	61.99	193
HPRT1	hypoxanthine phosphoribosyltransferase 1	ATAAGCCAGACTTTGTTGGA	605	20	59.23	CTCAACTTGAACTCTCATCTTAGG	760	24	60.76	155
RPL19	ribosomal protein L19	ATGAGTATGCTCAGGCTTCAG	62	21	61.99	GATCAGCCCATCTTTGATGAG	211	21	60.88	149

http://medgen.ugent.be/rtprimerdb

Method Side Population analysis

Immediately after surgical removal of the tumour (resection samples), part of the tissue was fixed in 6% formalin and embedded in paraffin (FFPE), while the other part was freshly cut in small blocks of 1mm by 1mm to be frozen overnight in Recovery™ Cell Culture Freezing Medium (Invitrogen, Carlsbad, CA, USA) on -80°C and subsequently stored in liquid nitrogen. The histopathological diagnosis of HCC was done according to the World Health Organization criteria and were were further subdivided into keratin (K) 19 negative and K19 positive HCCs based on immunohistochemical stainings for K19 (1/25; Dako, Glostrup, Denmark) on the FFPE samples. Frozen samples were thawed at 37°C, washed with Hank's balanced salt solution (Invitrogen, Carlsbad, CA, USA), and dissociated using Liberase Blendzyme 3 (Roche, Basel, Switzerland) at a concentration of 0.8 Wunsch unit/ml during 1,5 hours at 37°C. The samples were filtered using a 70 µm nylon mesh filter (BD Biosciences, Franklin Lakes NJ, USA) and a 15% Percoll/ Hanks' Balanced Salt Solution (100g for 15min). The cells were finally resuspended in Hepatozyme-SFM, containing 1% Penicillin/Streptomycin (Invitrogen) and incubated with 5 µg/ml Hoechst33342 (Sigma-Aldrich, St Louis MO, USA) for exactly 90 min at 37°C under continuous agitation. To assess active efflux and side population phenotype, cells were incubated with the transport blockers verapamil (100 µM; Sigma-Aldrich), 20 minutes prior to the Hoechst33342 incubation. Propidium iodide (2 µg/ml; Sigma-Aldrich) was added to exclude dead cells. The cell suspensions were analysed using a FACSAriaII (BD Biosciences). The SP was visualized after UV excitation on the basis of blue emission through a 424/44 filter and of red emission through a 630/22 filter (Omega Optical, Brattleboro, VT). Within the living cell population (propidium iodide negative), the side and main population (MP) were sorted separately and collected in Hepatozyme-SFM. Part of the sorted cells was fixated in BD CytoRich™ System (BD Biosciences) and processed into cytospins. Immunohistochemistry was performed on the cytospins to assess K19 expression in the sorted samples. Statistical analysis to determine significant differences in the size of the Side Population fraction was performed using the non-parametric Man-Whitney U test, with Graphpad Prism 5.02 (GraphPad Software, Inc., La Jolla, CA, USA). All analysis was two-tailed. In all cases, $p < 0.05$ was considered significant.