Autologous cell lines from circulating colon cancer cells captured from sequential liquid biopsies as model to study therapy-driven tumor changes

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Supplementary Table S1. Oncogene mutations analyzed by Imagenome™ colorectal cancer panel.

	Exons	Codons
KRAS	2	12, 13
	3	61
	4	146
NRAS	2	12, 13
	3	61
	4	146
BRAF	15	600
PIK3CA	9	539, 542, 545, 546
	20	1047, 1048, 1049
PTEN	7	159, 233, 267 (del)
AKT	4	17

Supplementary Table S2. Sequence of the forward and reverse primers used for qRT-PCR analysis

Gene	Entrez Gene Name	Location	Type	Oligo Name	Sequence 5' to 3'	
DEFA6	Defensin alpha 6	Extracellular Space	Other	Forward Sequence	ATGACCAGGACTTTGCCGTCTC	NM_001926
				Reverse Sequence	CATGACAGTGCAGGTCCCATAG	
BCL11A	B-cell CLL/lymphoma 11A	Nucleus	Transcription regulator	Forward Sequence	CTCGTTCTGCACATGGAGCTCT	NM_022893
				Reverse Sequence	GCAAGAGAAACCATGCACTGGTG	
IL33	Interleukin 33	Extracellular Space	Cytokine	Forward Sequence	GCCTGTCAACAGCAGTCTACTG	NM_033439
				Reverse Sequence	TGTGCTTAGAGAAGCAAGATACTC	
HS6ST2	Heparan sulfate 6-O-sulfotransferase 2	Plasma Membrane	Enzyme	Forward Sequence	CTCTCCGTCATCCACAAAGACC	NM_001077188
				Reverse Sequence	CAGGGATGCTTTCCATGTTGCC	
GJB6	Gap junction protein beta 6	Plasma Membrane	Transporter	Forward Sequence	GAAACCACTCGCAAGTTCAGGC	NM_006783
				Reverse Sequence	AGGCTGCTTCAAAGATGATTCGG	
SMARCA1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1	Nucleus	Transcription regulator	Forward Sequence	AACCTGGCAAGTGCTGATGTGG	NM_003069
				Reverse Sequence	CAGTGTTGTCAGTGATGAGACGG	
ADAMTS6	ADAM metallopeptidase with thrombospondin type 1 motif 6	Extracellular Space	Peptidase	Forward Sequence	GTGATCCTGACAGTAAGCCACC	NM_197941
				Reverse Sequence	CCACCATCACAAGTCTTGCTGC	
BMP7	Bone morphogenetic protein 7	Extracellular Space	Growth factor	Forward Sequence	GAGTGTGCCTTCCCTCTGAACT	NM_001719
				Reverse Sequence	AGGACGGAGATGGCATTGAGCT	
ABCB1	ATP binding cassette subfamily B member 1	Plasma Membrane	Transporter	Forward Sequence	GCTGTCAAGGAAGCCAATGCCT	NM_000927
				Reverse Sequence	TGCAATGGCGATCCTCTGCTTC	
CCND2	Cyclin D2	Nucleus	Other	Forward Sequence	GAGAAGCTGTCTCTGATCCGCA	NM_001759
				Reverse Sequence	CTTCCAGTTGCGATCATCGACG	
TNFRSF1B	TNF receptor superfamily member 1b	Plasma Membrane	Transmembrane receptor	Forward Sequence	CGTTCTCCAACACGACTTCATCC	NM_001066
				Reverse Sequence	ACGTGCAGACTGCATCCATGCT	
FN1	Fibronectin 1	Extracellular Space	Enzyme	Forward Sequence	ACAACACCGAGGTGACTGAGAC	NM_212482
				Reverse Sequence	GGACACAACGATGCTTCCTGAG	
SEMA6A	Semaphorin 6A	Plasma Membrane	Transmembrane receptor	Forward Sequence	ACCTGTATTGCCTCCAGAGACC	NM_020796
				Reverse Sequence	CCAGACCATCTGTATTGCCACG	
INHBB	Inhibin beta B subunit	Extracellular Space	Growth factor	Forward Sequence	GAAATCATCAGCTTCGCCGAGAC	NM_002193
				Reverse Sequence	GGCAGGAGTTTCAGGTAAAGCC	
GAL	Galanin and GMAP prepropeptide	Extracellular Space	Other	Forward Sequence	TGGCAACCACAGGTCATTCAGC	NM_015973
				Reverse Sequence	TCAGGTATGGACCTGTCAAAGCT	
PTGS2	Prostaglandin-endoperoxide synthase 2	Cytoplasm	Enzyme	Forward Sequence	CGGTGAAACTCTGGCTAGACAG	NM_000963
				Reverse Sequence	GCAAACCGTAGATGCTCAGGGA	
WNT11	Wnt family member 11	Extracellular Space	Other	Forward Sequence	CTGTGAAGGACTCGGAACTCGT	NM_004626
				Reverse Sequence	AGCTGTCGCTTCCGTTGGATGT	
TGFB2	Transforming growth factor beta 2	Extracellular Space	Growth factor	Forward Sequence	AAGAAGCGTGCTTTGGATGCGG	NM_003238
				Reverse Sequence	ATGCTCCAGCACAGAAGTTGGC	
DKK1	Dickkopf WNT signaling pathway inhibitor 1	Extracellular Space	Growth factor	Forward Sequence	GGTATTCCAGAAGAACCACCTTG	NM_012242
				Reverse Sequence	CTTGGACCAGAAGTGTCTAGCAC	
BST2	Bone marrow stromal cell antigen 2	Plasma Membrane	Other	Forward Sequence	TCTCCTGCAACAAGAGCTGACC	NM_004335
				Reverse Sequence	TCTCTGCATCCAGGGAAGCCAT	
GATA2	GATA binding protein 2	Nucleus	Transcription regulator	Forward Sequence	CAGCAAGGCTCGTTCCTGTTCA	NM_032638
				Reverse Sequence	ATGAGTGGTCGGTTCTGCCCAT	
EPCAM	Epithelial cell adhesion molecule	Plasma Membrane	Cell adhesion	Forward Sequence	ACAAGGACACTGAAATAACCTGC	NM_002354
				Reverse Sequence	TTTTGAGAAGAATTTTGAACCAGAT	
CK19	Keratin 19	Cytoplasm	Intermediate filament	Forward Sequence	GCGACTACAGCCACTACTACACG	NM_002276
				Reverse Sequence	CTTCGCATGTCACTCAGGATCT	
ECAD	Cadherin-1	Plasma Membrane	Cell adhesion	Forward Sequence	CCGAGAGCTACACGTTC	NM_004360
				Reverse Sequence	TCTTCAAAATTCACTCTGCC	
NCAD	Cadherin-2	Plasma Membrane	Cell adhesion	Forward Sequence	ACATATGTGATGACCGTAAC	NM_001792
				Reverse Sequence	TTTTTCTCGATCAAGTCCAG	
VIM	Vimentin	Cytoplasm	Intermediate filament	Forward Sequence	GGAAACTAATCTGGATTCACTC	NM_003380
				Reverse Sequence	CACTTCTAGTTTCAACCGTC	
OPG	TNF receptor superfamily member 11b	Plasma Membrane	Transmembrane receptor	Forward Sequence	GGTCTCCTGCTAACTCAGAAAGG	NM_002546
				Reverse Sequence	CAGCAAACCTGAAGAATGCCTCC	
VEGF	Vascular endothelial growth factor A	Extracellular Space	Growth factor	Forward Sequence	ATCTTCAAGCATCCTGTGT	NM_001025366.2
				Reverse Sequence	GCTCACCGCCTCGGCTTGT	
ALDH1	Aldehyde dehydrogenase 1 family member A1	Cytoplasm	Enzyme	Forward Sequence	CGGGAAAAGCAATCTGAAGAGGG	NM_000689
				Reverse Sequence	GATGCGGCTATACAACACTGGC	
CD133	Prominin-1	Plasma Membrane	Other	Forward Sequence	TTGAATGAAACTCCAGAGCAAA	NM_001145847
				Reverse Sequence	CCTCCTAGCACTGAATTGATACTG	
SOX2	SRY-box 2	Nucleus	Transcription regulator	Forward Sequence	AGCATGGAGAAAACCCGGTA	NM_003106
				Reverse Sequence	TTTTGCGTGAGTGTGGATGG	
TWIST	Twist-related protein	Nucleus	Transcription factor	Forward Sequence	GGCCGGAGACCTAGATGT	NM_000474
				Reverse Sequence	CACGCCCTGTTTCTTTGAAT	
SNAIL	Snail family transcriptional repressor	Nucleus	Transcription repressor	Forward Sequence	TGCCCTCAAGATGCACATCCGA	NM_005985
				Reverse Sequence	GGGACAGGAGAAGGGCTTCTC	
CEACAM1	Carcinoembryonic antigen related cell adhesion molecule 1	Plasma Membrane	Transporter	Forward Sequence	GAGTAGTGGCCCTGGTTGCTC	NM_001712
				Reverse Sequence	CGCTGGTCGCTTGCCCT	
CMET	MET proto-oncogene, receptor tyrosine kinase	Plasma Membrane	Transmembrane receptor	Forward Sequence	TTTATTAGTGGTGGGAGCACAAT	NM_001127500
				Reverse Sequence	GATGTTGACATGCCACTGTAAAG	
CD45	Protein tyrosine phosphatase, receptor type C	Plasma Membrane	Transmembrane receptor	Forward Sequence	TCACTTGCTCCTCAAACTGAGA	NM_002838
				Reverse Sequence	AGATGTACTGGGCCTCTACTTGA	
B2M	Beta-2-microglobulin	Plasma Membrane	Housekeeping gene	Forward Sequence	GTCTTTCAGCAAGGACTGGTCT	NM_004048
				Reverse Sequence	TTACATGTCTCGATCCCACTTAAC	

Supplementary Table S3. List of antibodies used for the CTC lines protein characterization.

Antibodies	Supplier	Clone	Ref	Conjugate	IgG
CD105	Miltenyi	43A4E1	130-094-941	PE	Mouse IgG1
CD133	Miltenyi	AC133	130-080-801	PE	Mouse IgG1
CD24	Miltenyi	32D12	130-095-953	PE	Mouse IgG1
PanCD66 (a,c,d,e)	Miltenyi	REA428	130-106-344	PE	Recombinant Human IgG1
PanCK (8,18,19)	Miltenyi	CK3-6H5	130-080-101	FITC	Mouse IgG1
EpCAM	Miltenyi	HEA-125	130-080-301	FITC	Mouse IgG1
CD146	Miltenyi	541-10B2	130-092-849	APC	Mouse IgG1
E-Cadherin	Miltenyi	67A4	130-095-412	APC	Mouse IgG1
CD44	Beckman Coulter	J.173	IM1219U	FITC	Mouse IgG1
CD45	Beckman Coulter	J.33	A07782	FITC	Mouse IgG1
CD34	Beckman Coulter	581	IM2709U	ECD	Mouse IgG1
CD309	Beckman Coulter	KDR-1	A64616	PC7	Mouse IgG1
CXCR-4	Beckman Coulter	12G5	A07409	PE	Mouse IgG2
CD31	Biolegend	WM59	303114	PB	Mouse IgG1
EGFR	Biolegend	AY13	352908	FITC	Mouse IgG1
N-cadherin	Biolegend	8C11	350805	PE	Mouse IgG1
CK19	Abcam	EP1508Y	Ab192643	A488	IgG
ALDH1	Abcam	EP1933Y	ab195254	A488	IgG
Her-2 neu	Abcam	24D2	ab481188	PE	IgG2a
Vimentin	BD Pharmingen	RV202	562337	PE	Mouse IgG1
CK20	Progen	IT-Ks20.10	61454	FITC	IgG1
PD-L1	R&D system	130021	FAB1561P	PE	Mouse IgG1
VEGF	R&D system	23410	IC2931G	A488	Mouse IgG2
CD44v6	R&D system	2F10	FAB3660A	APC	Mouse IgG1

Supplementary Table S4. CTC's DNA mutational status of all CTC lines for 6 relevant oncogenes in colorectal were analyzed by Mass-Array

		CTC-MCC-41	CTC-MCC-41.4	CTC-MCC-41.5A	CTC-MCC-41.5B	CTC-MCC-41.5C	CTC-MCC-41.5D	CTC-MCC-41.5E	CTC-MCC-41.5F	CTC-MCC-41.5G
KRAS	**Exon 2**	WT	WT	WT	WT	WT	WT	WT	WT	WT
	Exon 3	WT	WT	WT	WT	WT	WT	WT	WT	WT
	Exon 4	WT	WT	WT	WT	WT	WT	WT	WT	WT
NRAS	**Exon 2**	WT	WT	WT	WT	WT	WT	WT	WT	WT
	Exon 3	WT	WT	WT	WT	WT	WT	WT	WT	WT
	Exon 4	WT	WT	WT	WT	WT	WT	WT	WT	WT
BRAF	**Exon 15**	*c.1799T>A; p.V600E 50%*	*c.1799T>A; p.V600E 50%*	*c.1799T>A; p.V600E 50%*	*c.1799T>A; p.V600E 50%*	*c.1799T>A; p.V600E 50%*	*c.1799T>A; p.V600E 50%*	*c.1799T>A; p.V600E 50%*	*c.1799T>A; p.V600E 50%*	*c.1799T>A; p.V600E 50%*
PIK3CA	**Exon 9**	WT	WT	WT	WT	WT	WT	WT	WT	WT
	Exon 20	WT	WT	WT	WT	WT	WT	WT	WT	WT
PTEN	**Exon 7**	WT	WT	WT	WT	WT	WT	WT	WT	WT
AKT	**Exon 4**	WT	WT	WT	WT	WT	WT	WT	WT	WT

Supplementary Table S5. Clinical data of the 044 COLOSPOT patient at baseline.

Inclusion number	**01-044**	
Age	57	
Gender	Male	
Cancer localization	Transverse colon	
TNM classification	pTx pNx pM1	
Metastatic sites		
Number	2	
Localization	Liver	Lymph nodes
Size	1* 22mm	Multiple* ≥15mm
Tumor markers		
CA19.9	106.3 U/ml	
ACE	3.8 ng/ml	
Mutations		
KRAS	Wild type	
BRAF	V600E mutation	

Supplementary Table S6. Normalized quantification of mRNAs expression in all colon CTC lines, HT-29, SW620 and leukocytes.

		CTC-MCC-41	CTC-MCC-41.4	CTC-MCC-41.5A	CTC-MCC-41.5B	CTC-MCC-41.5C	CTC-MCC-41.5D	CTC-MCC-41.5E	CTC-MCC-41.5F	CTC-MCC-41.5G	HT-29	SW620	Leukocytes
CTC-MCC, HT-29 & SW620	***CK19***	154.83	31.52	55.31	33.15	28.51	23.31	105.38	34.95	71.48	43.83	107.92	*0*
	EPCAM	6.72	29.84	40.61	53.38	12.93	16.71	165.62	38.80	16.78	36.45	69.73	*0*
	ECAD	0.22	0.36	0.27	0.42	0.08	0.59	0.56	1.05	1.16	0.48	1.9	*0*
	VEGF	9.74	4.46	40.30	64.13	5.14	36.31	12.66	58.19	20.53	2.08	7.64	*0*
	CMET	31.14	3.86	8.74	34.25	0.77	2.51	42.35	5.63	6.50	7.70	12.5	*0*
	SNAIL	1.41	0.45	2.11	3.88	3.84	2.33	8.04	17.41	11.00	0.05	3.82	*0*
	ALDH1	13.22	2.49	47.12	14.70	41.42	7.24	18.35	60.17	43.33	58.73	0.30	*0*
	CEACAM1	2.40	2.97	6.15	3.47	1.87	1.23	1.43	4.96	1.30	2.16	0.31	*0*
	NCAD	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
	CD45	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	4.52
CTC-MCC vs HT-29 & SW620	***IL33***	30.56	26.83	1.70	6.65	166.16	36.59	24.67	3.32	0.32	*0*	*0*	*0*
	SEMA6A	10.04	95.07	1.36	69.40	1.17	0.47	3.53	2.07	0.70	*0*	*0*	*0*
	CCND2	9.82	63.35	0.05	0.42	14.10	1.97	3.47	0.17	0.11	*0*	*0*	*0*
	OPG	1.14	3.72	10.01	11.89	25.74	1.20	0.43	39.59	36.90	*0*	*0*	*0*
	SMARCA1	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	6.10	5.67	*0*
	DKK1	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	1.73	3.79	*0*
CTC-MCC & SW620 vs HT-29	***ADAMTS6***	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0.61	*0*	*0*
	GATA2	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	5.55	*0*	*0*
	GJB6	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0.45	*0*	*0*
	TGFB2	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	4.21	*0*	*0*
	PTGS2	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	6.33	*0*	*0*
	WNT11	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	1.11	*0*	*0*
	BST2	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	2.77	*0*	*0*
	TWIST	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	15.93	*0*	*0*
	BCL11A	1.70	3.93	9.67	5.98	0.89	0.62	1.29	2.812	2.42	*0*	2.09	*0*
	BMP7	8.40	12.09	19.33	15.85	9.06	3.16	5.86	13.73	11.92	*0*	27.16	*0*
	FN1	0.05	142.26	1.16	31.87	2.17	0.71	5.35	3.96	1.11	*0*	2.25	*0*
	TNFRSF1B	3.00	35.51	0.11	2.21	0.87	0.64	0.14	0.02	0.024	*0*	2.57	*0*
CTC-MCC & HT-29 vs SW620	***SOX2***	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	1.88	*0*
	VIM	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	143.45	21.93
CTC-MCC differences	***DEFA6***	73.31	17.10	*0*	*0*	930.82	45.96	4.91	*0*	0.30	*0*	*0*	*0*
	ABCB1	56.22	57.14	*0*	6.48	2.86	2.89	5.13	*0*	0.02	*0*	1.59	*0*
	GAL	2.45	*0*	*0*	0	0.87	0.03	0.15	*0*	0.02	*0*	5.79	*0*

Supplementary Table S7. Proteins expression by the 9 colon CTC lines using Flow cytometry.

	CTC-MCC-41	CTC-MCC-41.4	CTC-MCC-41.5A	CTC-MCC-41.5B	CTC-MCC-41.5C	CTC-MCC-41.5D	CTC-MCC-41.5E	CTC-MCC-41.5F	CTC-MCC-41.5G
E-cadherin	Low	low	low	low	+	+	+	+	+
CK20	++	+ +	+ +	+ +	+ +	+ +	+ +	+	+
PanCK	++	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
CD31	-	-	-	-	-	-	-	-	-
CD146	-	-	-	-	-	-	-	-	-
CD105	-	-	-	-	-	-	-	-	-
CD34	-	-	-	-	-	-	-	-	-
ALDH1	-	-	-	-	-	-	-	-	-
CD309	-	-	-	-	-	-	-	-	-
PD-L1	-	-	-	-	-	-	-	-	-
CXCR4	-	-	-	-	-	-	-	-	-
HER-2 neu	-	-	-	-	-	-	-	-	-
FGF-2	-	-	-	-	-	-	-	-	-
OPG	-	-	-	-	-	-	-	-	-