

Heterogeneity between primary colon carcinoma and paired lymphatic and hepatic metastases

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Abstract. Heterogeneity is one of the recognized characteristics of human tumors, and occurs on multiple levels in a wide range of tumors. A number of studies have focused on the heterogeneity found in primary tumors and related metastases with the consideration that the evaluation of metastatic rather than primary sites could be of clinical relevance. Numerous studies have demonstrated particularly high rates of heterogeneity between primary colorectal tumors and their paired lymphatic and hepatic metastases. It has also been proposed that the heterogeneity between primary colon carcinomas and their paired lymphatic and hepatic metastases may result in different responses to anticancer therapies. The heterogeneity in primary colon carcinoma and corresponding metastases by genome-wide gene expression analysis has not been extensively studied. In the present study, we investigated the differentially expressed genes between a primary colon carcinoma specimen (obtained from a 40-year-old female colon carcinoma patient with lymphatic and hepatic metastases) and its paired lymphatic and hepatic metastases by genome-wide gene expression analysis using GeneChip HGU133Plus2.0 expression arrays. Our results demonstrate that genome-wide gene expression varies between primary colon carcinoma and its paired lymphatic and hepatic metastases.

Introduction

Intratumor heterogeneity is one of the recognized characteristics of human tumors, which occurs on multiple levels, including genetic, protein and macroscopic, in a wide range of tumors, including breast, colorectal cancer (CRC), non-small cell lung cancer (NSCLC), prostate, ovarian, pancreatic, gastric, brain and renal clear cell carcinoma (1). Over the past decade, a number of studies have focused on the heterogeneity found in primary tumors and related metastases with the consideration that the evaluation of metastatic rather than primary sites may be of clinical relevance. Numerous reports have evaluated the genetic heterogeneity in primary tumors and corresponding metastases in a range of solid tumors, including breast cancer (2-9), CRC (10-13) and NSCLC (14,15).

In CRC, the heterogeneity of the epidermal growth factor receptor (*EGFR*) gene status and its downstream signalling proteins, including *KRAS*, *BRAF* and *PIK3CA* mutations, have been identified by comparing primary tumors with corresponding metastases, respectively (10-13). Baldus *et al* demonstrated particularly high rates of heterogeneity between primary colorectal tumors and lymph node metastases (10). Molinari *et al* analyzed *EGFR* gene status and protein expression, *KRAS/BRAF* mutations and PTEN expression in primary tumors and metastases in 38 metastatic CRC patients, and found *EGFR* gene deregulation in 69.4% of primary tumors and 80.6% of metastases, and *KRAS* mutations in 43.2% of primary tumors and 40.5% of metastases (11). However, the heterogeneity in primary CRC and corresponding metastases at the genome-wide gene expression level has not been extensively investigated.

Therefore, in the present study, we investigated the heterogeneity in primary colon carcinoma tissue and its corresponding lymphatic and hepatic metastatic tissues obtained from a female metastatic colon cancer patient, focusing on genome-wide gene expression.

Patients and methods

Patient and tissue samples. Tumor specimens were obtained at initial surgery from a 40-year-old female colon carcinoma

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Key words: colon carcinoma, hepatic metastasis, heterogeneity, genome-wide gene expression, functional gene

Table I. Genes differentially expressed in the primary colon carcinoma specimen and its paired lymphatic metastasis.

Probe set ID	Fold change	Gene symbol	Entrez gene	Gene title
201847_at	23.27895	LIPA	3988	Lipase A, lysosomal acid, cholesterol esterase
203323_at	20.588184	CAV2	858	Caveolin 2
203324_s_at	25.194075	CAV2	858	Caveolin 2
203571_s_at	23.1392	C10 or f116	10974	Chromosome 10 open reading frame 116
203639_s_at	69.52935	FGFR2	2263	Fibroblast growth factor receptor 2
203697_at	20.352211	FRZB	2487	Frizzled-related protein
204010_s_at	34.406235	KRAS	3845	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
204035_at	39.844757	SCG2	7857	Secretogranin II (chromogranin C)
204439_at	46.355186	IFI44L	10964	Interferon-induced protein 44-like
204802_at	113.641525	RRAD	6236	Ras-related associated with diabetes
204803_s_at	41.770977	RRAD	6236	Ras-related associated with diabetes
205151_s_at	36.290764	KIAA0644	9865	KIAA0644 gene product
205625_s_at	29.481396	CALB1	793	Calbindin 1, 28 kDa
205696_s_at	45.68169	GFRA1	2674	GNDF family receptor α 1
205778_at	28.692463	KLK7	5650	Kallikrein-related peptidase 7
205818_at	109.04431	DBC1	1620	Deleted in bladder cancer 1
205830_at	20.343859	CLGN	1047	Calmegin
206091_at	25.613195	MATN3	4148	Matrilin 3
206340_at	24.728613	NR1H4	9971	Nuclear receptor subfamily 1, group H, member 4
206525_at	25.197699	GABRR1	2569	γ -aminobutyric acid (GABA) receptor, rho 1
206619_at	34.690308	DKK4	27121	Dickkopf homolog 4 (<i>Xenopus laevis</i>)
206877_at	20.351955	MXD1	4084	MAX dimerization protein 1
207254_at	48.329205	SLC15A1	6564	Solute carrier family 15 (oligopeptide transporter), member 1
207509_s_at	51.56186	LAIR2	3904	Leukocyte-associated immunoglobulin-like receptor 2
207583_at	40.26811	ABCD2	225	ATP-binding cassette, sub-family D (ALD), member 2
207610_s_at	23.911833	EMR2	30817	Egf-like module containing, mucin-like, hormone receptor-like 2
208146_s_at	89.68937	CPVL	54504	Carboxypeptidase, vitellogenic-like
208261_x_at	29.10015	IFNA10	3446	Interferon, α 10
208481_at	26.099915	ASB4	51666	Ankyrin repeat and SOCS box-containing 4
208791_at	46.167988	CLU	1191	Clusterin
209406_at	29.201277	BAG2	9532	BCL2-associated athanogene 2
209792_s_at	38.65837	KLK10	5655	Kallikrein-related peptidase 10
209821_at	205.30872	IL33	90865	Interleukin 33
210510_s_at	27.80813	NRP1	8829	Neuropilin 1
210931_at	24.963585	RNF6	6049	Ring finger protein (C3H2C3 type) 6
211161_s_at	30.307695	COL3A1	1281	Collagen, type III, α 1
211401_s_at	20.871784	FGFR2	2263	Fibroblast growth factor receptor 2
213238_at	42.231457	ATP10D	57205	ATPase, class V, type 10D
213428_s_at	44.14465	COL6A1	1291	Collagen, type VI, α 1
213432_at	189.0725	MUC5B	727897	Mucin 5B, oligomeric mucus/gel-forming
213874_at	60.066086	SERPINA4	5267	Serpin peptidase inhibitor, clade A (α -1 antitrypsin), member 4
214044_at	23.23853	RYR2	6262	Ryanodine receptor 2 (cardiac)
214121_x_at	28.547356	PDLIM7	9260	PDZ and LIM domain 7 (enigma)
214146_s_at	40.065388	PPBP	5473	Pro-platelet basic protein [chemokine (C-X-C motif) ligand 7]
214453_s_at	33.425632	IFI44	10561	Interferon-induced protein 44
214567_s_at	25.704966	XCL1	6375	Chemokine (C motif) ligand 1
		XCL2	6846	Chemokine (C motif) ligand 2
214769_at	31.164827	CLCN4	1183	Chloride channel 4
214811_at	62.246216	RIMBP2	23504	RIMS binding protein 2
214823_at	36.446995	ZNF204	7754	Zinc finger protein 204 pseudogene
214952_at	31.43681	NCAM1	4684	Neural cell adhesion molecule 1
215081_at	38.364174	KIAA1024	23251	KIAA1024
215318_at	27.675882	CG012	116829	Hypothetical gene CG012
215917_at	27.72305	SNPH	9751	Syntrophin
216039_at	28.136038	LOC100132832	100132832	Postmeiotic segregation increased 2-like 5-like
		LOC441259	392713	PMS2 postmeiotic segregation increased 2 (<i>S. cerevisiae</i>)-like
		LOC730324	441259	Similar to postmeiotic segregation increased 2-like 2
		PMS2	5379	PMS2 postmeiotic segregation increased 2 (<i>S. cerevisiae</i>)
		PMS2L1	5380	Postmeiotic segregation increased 2-like 1 pseudogene

Table I. Continued.

Probe set ID	Fold change	Gene symbol	Entrez gene	Gene title
		PMS2L12	5383	Postmeiotic segregation increased 2-like 12 pseudogene
		PMS2L2	5387	Postmeiotic segregation increased 2-like 2 pseudogene
		PMS2L3	5395	Postmeiotic segregation increased 2-like 3
		PMS2L5	730324	Postmeiotic segregation increased 2-like 5
216614_at	38.53543			
217546_at	59.020702	MT1M	4499	Metallothionein 1M
217664_at	26.640495			
218720_x_at	44.51107	LOC652900	26470	Similar to seizure related 6 homolog (mouse)-like 2 isoform 1
		SEZ6L2	652900	Seizure related 6 homolog (mouse)-like 2
219396_s_at	41.20278	NEIL1	79661	Nei endonuclease VIII-like 1 (<i>E. coli</i>)
219563_at	28.401752	C14 or f139	79686	Chromosome 14 open reading frame 139
219727_at	29.042574	DUOX2	50506	Dual oxidase 2
219728_at	23.185019	MYOT	9499	Myotilin
219768_at	90.29275	VTCN1	79679	V-set domain containing T cell activation inhibitor 1
219947_at	43.223457	CLEC4A	50856	C-type lectin domain family 4, member A
219949_at	53.802288	LRRC2	79442	Leucine-rich repeat containing 2
220086_at	24.022339	IKZF5	64376	IKAROS family zinc finger 5 (Pegasus)
220160_s_at	20.245548	KPTN	11133	Kaptin (actin binding protein)
221195_at	25.616514	RNFT1	51136	Ring finger protein, transmembrane 1
222106_at	26.959286	PRND	23627	Prion protein 2 (dublet)
222565_s_at	60.40278	PRKD3	23683	Protein kinase D3
222668_at	114.43788	KCTD15	79047	Potassium channel tetramerisation domain containing 15
222720_x_at	23.61017	C1 or f27	54953	Chromosome 1 open reading frame 27
223235_s_at	76.54248	SMOC2	64094	SPARC related modular calcium binding 2
223599_at	21.029135	TRIM6	117854	Tripartite motif-containing 6
223985_at	27.464828	FSD1L	83856	Fibronectin type III and SPRY domain containing 1-like
223999_at	26.675077	PPIL2	23759	Peptidylprolyl isomerase (cyclophilin)-like 2
224098_at	32.526546			
224348_s_at	34.73594			
224533_s_at	77.99536	C6 or f142	90523	Chromosome 6 open reading frame 142
225627_s_at	23.322224	CACHD1	57685	Cache domain containing 1
225728_at	26.672054	SORBS2	8470	Sorbin and SH3 domain containing 2
226591_at	26.339521	SNRPN	6638	Small nuclear ribonucleoprotein polypeptide N
227491_at	20.302715	ELOVL6	79071	ELOVL family member 6, elongation of long chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast)
227870_at	48.255272	IGDCC4	57722	Immunoglobulin superfamily, DCC subclass, member 4
227925_at	65.23458	FLJ39051	399972	Hypothetical gene supported by AK096370
227955_s_at	29.176859	EFNA5	1946	Ephrin-A5
228007_at	20.671658	C6 or f204	387119	Chromosome 6 open reading frame 204
228072_at	35.00761	SYT12	91683	Synaptotagmin XII
229228_at	178.89961	CREB5	9586	cAMP responsive element binding protein 5
229450_at	20.979269	IFIT3	3437	Interferon-induced protein with tetratricopeptide repeats 3
229478_x_at	48.66807	BIVM	54841	Basic, immunoglobulin-like variable motif containing
229518_at	24.889814	FAM46B	115572	Family with sequence similarity 46, member B
229579_s_at	42.72015	DISP2	85455	Dispatched homolog 2 (<i>Drosophila</i>)
229725_at	30.584335	ACSL6	23305	Acyl-CoA synthetase long-chain family member 6
230245_s_at	25.038433	LOC283663	283663	Hypothetical LOC283663
230487_at	66.86591	C6 or f99	100130967	Chromosome 6 open reading frame 99
		LOC100130967	389440	Similar to hCG2044932
230615_at	46.127205	DUOXA2	405753	Dual oxidase maturation factor 2
230660_at	37.561954	SERTAD4	56256	SERTA domain containing 4
230781_at	42.71768			
231102_at	32.751915	CROT	54677	Carnitine O-octanoyltransferase
231203_at	35.097168	LOC100129633	100129633	Similar to hCG1651427
231336_at	55.55161	CPNE4	131034	Copine IV
231598_x_at	111.41086			
231899_at	30.031355	ZC3H12C	85463	Zinc finger CCCH-type containing 12C
232068_s_at	20.96932	TLR4	7099	Toll-like receptor 4
232387_at	23.740568	AP1GBP1	11276	AP1 γ subunit binding protein 1
232585_at	24.154518	LOC100128729	100128729	Similar to TLK2 protein

Table I. Continued

Probe set ID	Fold change	Gene symbol	Entrez gene	Gene title
		LOC100134651	100134651	Similar to TLK2 protein
		LOC731082	11011	Similar to serine/threonine-protein kinase tousled-like 2 (tousled-like kinase 2)
		TLK2	731082	Tousled-like kinase 2
233149_at	20.532196			
233372_at	48.244698			
233884_at	21.642752	HIVEP3	59269	Human immunodeficiency virus type I enhancer binding protein 3
				Guanylate cyclase activator 1C
233902_at	35.82076	GUCA1C	9626	
233984_at	22.362148			
234700_s_at	146.27199	RNASE7	84659	Ribonuclease, RNase A family, 7
234980_at	55.37136	TMEM56	148534	Transmembrane protein 56
235795_at	27.020695	PAX6	5080	Paired box 6
236302_at	21.677221	PPM1E	22843	Protein phosphatase 1E (PP2C domain containing)
236365_at	31.352936			
236522_at	25.091562			
236542_at	42.33878			
236740_at	25.037415			
236801_at	50.310337			
237250_at	49.111843			
237406_at	28.270689	NEK6	10783	NIMA (never in mitosis gene a)-related kinase 6
237950_s_at	49.878513			
237974_at	36.01243	ABHD12B	145447	Abhydrolase domain containing 12B
237996_at	54.733566			
238088_at	20.880829	LOC100129194	100129194	Hypothetical protein LOC100129194
238363_at	29.599056			
238520_at	45.876377	TRERF1	55809	Transcriptional regulating factor 1
238611_at	22.46198			
239381_at	61.001457	KLK7	5650	Kallikrein-related peptidase 7
240214_at	27.089334			
241044_x_at	37.12408			
241579_at	40.58749			
241814_at	28.057734			
241842_x_at	27.38526	C19 or f45	374877	Chromosome 19 open reading frame 45
242003_at	35.487854	ERICH1	157697	Glutamate-rich 1
242138_at	27.004044	DLX1	1745	Distal-less homeobox 1
242249_at	21.104935			
242316_at	25.703829			
242509_at	26.935835			
242769_at	26.859947			
242791_at	26.823769	FBXO3	26273	F-box protein 3
242893_at	28.790308			
243753_at	20.372232	C1 or f173	127254	Chromosome 1 open reading frame 173
244016_at	41.27944			
244639_at	20.593792			
244785_at	25.557535			
1552480_s_at	27.07572	PTPRC	5788	Protein tyrosine phosphatase, receptor type, C
1552580_at	30.773138	TRIML2	205860	Tripartite motif family-like 2
1552904_at	26.276854	NETO1	81832	Neuropilin (NRP) and tolloid (TLL)-like 1
1552987_a_at	41.45497	MGC42157	439933	Hypothetical locus MGC42157
1553037_a_at	22.476143	SYN2	6854	Synapsin II
1553315_at	22.418777	SLFNL1	200172	Schlafen-like 1
1553635_s_at	22.641033	TCTEX1D1	200132	Tctex1 domain containing 1
1553789_a_at	27.130203	C21 or f58	54058	Chromosome 21 open reading frame 58
1554001_at	49.381943	TRIM37	4591	Tripartite motif-containing 37
1554398_at	27.990637	LYG2	254773	Lysozyme G-like 2
1554512_a_at	40.911217	CCDC123	84902	Coiled-coil domain containing 123
1555300_a_at	25.510637	MED12L	116931	Mediator complex subunit 12-like
1555717_at	38.556057			
1555912_at	33.604454	ST7OT1	93653	ST7 overlapping transcript 1 (non-protein coding)
1556008_a_at	36.32589			
1556202_at	39.568478	SRGAP2	23380	SLIT-ROBO Rho GTPase activating protein 2
1556541_s_at	29.452276			

Table I. Continued.

Probe set ID	Fold change	Gene symbol	Entrez gene	Gene title
1558552_s_at	20.966536	C3 or f55	152078	Chromosome 3 open reading frame 55
1558234_at	31.376406	FLJ36644	400617	Hypothetical protein LOC400617
1558791_at	43.679718	LOC286467	286467	Hypothetical LOC286467
1561114_a_at	22.05421	DEPDC4	120863	DEP domain containing 4
1560048_at	27.137468			
1561101_at	35.96605	LOC153469	153469	Hypothetical protein LOC153469 1561260_at 23.416117
1561573_at	23.90905			
1561714_a_at	41.711376	B3GALNT2	148789	β -1,3-N-acetylgalactosaminyltransferase 2
1561731_at	49.069042			
1561918_at	21.02261			
1562860_at	40.932205			
1562990_at	26.946367			
1563392_at	23.033009			
1563456_at	25.698204	CCDC141	285025	Coiled-coil domain containing 141
		LOC285026	285026	Hypothetical protein LOC285026
1564653_s_at	35.795006	LEKR1	389170	Leucine, glutamate and lysine rich 1
1564932_at	21.246243			
1565337_at	22.219374	DNAH6	1768	Dynein, axonemal, heavy chain 6
1568941_a_at	24.125954			
1569408_at	27.472164	EIF2C4	192670	Eukaryotic translation initiation factor 2C, 4
1569599_at	47.001877	SAMSN1	64092	SAM domain, SH3 domain and nuclear localization signals 1
1569772_x_at	23.892517			
1569832_at	34.25927	hCG_2045206	100131655	Hypothetical LOC100131655
1569948_at	21.763708			
1570076_at	30.77381			

patient with lymphatic and hepatic metastases. Samples were snap-frozen and stored in liquid nitrogen until use. Prior written informed consent was obtained from the patient and the study received ethics board approval at the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China. The patient had not received chemotherapy or radiation therapy prior to surgery. The histological type was determined according to the WHO criteria. The tumor was diagnosed as mucinous adenocarcinoma (T3N2M1).

Genome-wide gene expression analysis. RNA was extracted from tumor specimens. Total RNA of homogenized tumor samples was prepared with TRIzol RNA extraction reagent (Invitrogen) followed by purification using the RNeasy Mini kit (Qiagen) according to the manufacturer's instructions. A DNase I (Qiagen) digestion step was included to eliminate genomic DNA. The quality of the total RNA was examined for integrity using RNA LabChips and the Agilent Bioanalyzer 2100 (Agilent Technologies), and the concentration was measured using the Peqlab NanoDrop. Only RNA with an RNA integrity number >6.5 was used for cDNA synthesis. The one-cycle eukaryotic target labeling assay from Affymetrix was used according to manufacturer's instructions as previously described (16,17). Data analysis was also performed as previously described (16,17).

Statistical analysis. Hierarchical clustering of all microarray experiments was performed based on all 54,675 probe sets represented on the HGU133Plus2.0 array (Affymetrix)

($P < 0.04$) using positive correlation and complete linkage. Gene expression of the primary tumor was compared with the median arrays of the paired lymphatic metastatic and hepatic metastatic tumors from each xenograft model in a paired t-test.

Results

Heterogeneity in primary colon carcinoma and its paired lymphatic and hepatic metastases. Based on the results of the genome-wide gene expression analysis, a paired t-test between the primary colon carcinoma and its paired lymphatic metastasis revealed 190 differentially expressed probe sets with a fold change ≥ 20 . Clustering based on these 190 probe sets demonstrated a clear distinction between primary colon carcinoma and its paired lymphatic metastasis, with the majority of the probe sets (115 of 190) being downregulated in the lymphatic metastasis and only a small number being upregulated (75 of 190) (Table I). A paired t-test between the primary colon carcinoma and its paired hepatic metastasis revealed 150 differentially expressed probe sets with a fold change ≥ 20 . Clustering based on these 150 probe sets demonstrated a clear distinction between primary colon carcinoma and its paired hepatic metastasis, with the majority of probe sets (94 of 150) being downregulated in the hepatic metastasis and only a very small number being upregulated (56 of 150) (Table II). A paired t-test between colon carcinoma lymphatic metastasis and hepatic metastasis revealed 176 differentially expressed probe sets with a fold change of only ≥ 10 . Clustering based on these

Table II. Genes differentially expressed in the primary colon carcinoma specimen and its paired hepatic metastasis.

Probe set ID	Fold change	Gene symbol	Entrez gene	Gene title
201744_s_at	109.43063	LUM	4060	Lumican
203639_s_at	39.816193	FGFR2	2263	Fibroblast growth factor receptor 2
203687_at	23.863962	CX3CL1	6376	Chemokine (C-X3-C motif) ligand 1
203697_at	55.182484	FRZB	2487	Frizzled-related protein
203817_at	36.81657	GUCY1B3	2983	Guanylate cyclase 1, soluble, β 3
204010_s_at	40.820312	KRAS	3845	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
204439_at	50.40096	IFI44L	10964	Interferon-induced protein 44-like
204646_at	73.7989	DPYD	1806	Dihydropyrimidine dehydrogenase
204704_s_at	30.57162	ALDOB	229	Aldolase B, fructose-bisphosphate
205830_at	40.507374	CLGN	1047	Calmegin
205893_at	33.37443	NLGN1	22871	Neurologin 1
206091_at	43.29831	MATN3	4148	Matrilin 3
206218_at	29.100803	MAGEB2	4113	Melanoma antigen family B, 2
207349_s_at	24.040384	UCP3	7352	Uncoupling protein 3 (mitochondrial, proton carrier)
207583_at	27.094574	ABCD2	225	ATP-binding cassette, sub-family D (ALD), member 2
207750_at	29.539001	EPS15L2	55380	Epidermal growth factor receptor pathway substrate 15-like 2
208250_s_at	34.10199	DMBT1	1755	Deleted in malignant brain tumors 1
210077_s_at	23.053602	SFRS5	6430	Splicing factor, arginine/serine-rich 5
210143_at	36.192024	ANXA10	11199	Annexin A10
210510_s_at	52.630417	NRP1	8829	Neuropilin 1
210571_s_at	69.8555	CMAH	8418	Cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMP-N-acetylneuraminate monooxygenase) pseudogene
211204_at	21.602095	ME1	4199	Malic enzyme 1, NADP(+)-dependent, cytosolic
211842_s_at	20.652378	SLC24A1	9187	Solute carrier family 24 (sodium/potassium/calcium exchanger), member 1
212768_s_at	68.42675	OLFM4	10562	Olfactomedin 4
213496_at	31.919434	LPPR4	9890	Plasticity related gene 1
213519_s_at	26.87308	LAMA2	3908	Laminin, α 2
214044_at	35.559616	RYR2	6262	Ryanodine receptor 2 (cardiac)
214121_x_at	29.512691	PDLIM7	9260	PDZ and LIM domain 7 (enigma)
214146_s_at	50.72601	PPBP	5473	Pro-platelet basic protein [chemokine (C-X-C motif) ligand 7]
214567_s_at	24.816338	XCL1	6375	Chemokine (C motif) ligand 1
		XCL2	6846	Chemokine (C motif) ligand 2
214823_at	45.280537	ZNF204	7754	Zinc finger protein 204 pseudogene
215172_at	35.013386	PTPN20A	26095	Protein tyrosine phosphatase, non-receptor type 20A
		PTPN20B	653129	Protein tyrosine phosphatase, non-receptor type 20B
216236_s_at	22.056973	SLC2A14	144195	Solute carrier family 2 (facilitated glucose transporter)
		SLC2A3	6515	Member 14/solute carrier family 2 (facilitated glucose transporter) member 3
216918_s_at	22.751093	DST	667	Dystonin
217549_at	33.947136			
217584_at	28.199327			
219396_s_at	51.44716	NEIL1	79661	Nei endonuclease VIII-like 1 (<i>E. coli</i>)
219938_s_at	38.966156	PSTPIP2	9050	Proline-serine-threonine phosphatase interacting protein 2
219947_at	42.138695	CLEC4A	50856	C-type lectin domain family 4, member A
219949_at	40.146168	LRRC2	79442	Leucine-rich repeat containing 2
220253_s_at	49.8748	LRP12	29967	Low density lipoprotein-related protein 12
220333_at	24.75799	PAQR5	54852	Progestin and adipoQ receptor family member V
220858_at	40.026302	SORBS2	8470	Sorbin and SH3 domain containing 2
222249_at	32.831154			
222664_at	57.42625	KCTD15	79047	Potassium channel tetramerisation domain containing 15
222720_x_at	37.01965	C1 or f27	54953	Chromosome 1 open reading frame 27
222738_at	49.0615	WWC2	80014	WW and C2 domain containing 2
223235_s_at	83.11333	SMOC2	64094	SPARC related modular calcium binding 2
223553_s_at	60.20032	DOK3	79930	Docking protein 3
223645_s_at	23.843172	CYorf15B	84663	Chromosome Y open reading frame 15B
223985_at	25.144203	FSDIL	83856	Fibronectin type III and SPRY domain containing 1-like
223999_at	43.358845	PPIL2	23759	Peptidylprolyl isomerase (cyclophilin)-like 2
224029_x_at	53.921314	SCN11A	11280	Sodium channel, voltage-gated, type XI, α subunit
224135_at	28.728426	WDR87	83889	WD repeat domain 87
224370_s_at	51.475292	CAPS2	84698	Calcyphosine 2
224533_s_at	75.15053	C6 or f142	90523	Chromosome 6 open reading frame 142
225627_s_at	20.237694	CACHD1	57685	Cache domain containing 1

Table II. Continued.

Probe set ID	Fold change	Gene symbol	Entrez gene	Gene title
225728_at	45.161	SORBS2	8470	Sorbin and SH3 domain containing 2
226147_s_at	64.73383	PIGR	5284	Polymeric immunoglobulin receptor
226425_at	21.041115	CLIP4	79745	CAP-GLY domain containing linker protein family, member 4
226435_at	23.594818	PAPLN	89932	Papilin, proteoglycan-like sulfated glycoprotein
226591_at	29.041143	SNRPN	6638	Small nuclear ribonucleoprotein polypeptide N
227826_s_at	47.852997			
227925_at	29.181805	FLJ39051	399972	Hypothetical gene supported by AK096370
228007_at	23.74942	C6orf204	387119	Chromosome 6 open reading frame 204
228255_at	23.693722	ALS2CR4	65062	Amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 4
228766_at	35.04908	CD36	948	CD36 molecule (thrombospondin receptor)
229495_at	24.522652	PM20D2	135293	Peptidase M20 domain containing 2
230245_s_at	35.8261	LOC283663	283663	Hypothetical LOC283663
230720_at	20.412619	RNF182	221687	Ring finger protein 182
231102_at	126.71907	CROT	54677	Carnitine O-octanoyltransferase
231114_at	49.288345	SPATA22	84690	Spermatogenesis associated 22
231385_at	37.57739	DPPA3	359787	Developmental pluripotency associated 3
231560_at	24.556393			
231969_at	32.270084	STOX2	56977	Storkhead box 2
232049_at	20.035376			
232090_at	21.041267	LOC100128178	100128178	Similar to hCG2041313
232397_at	116.284195			
232481_s_at	28.357405	SLITRK6	84189	SLIT and NTRK-like family, member 6
232641_at	30.381804	ZNF596	169270	Zinc finger protein 596
233721_x_at	38.845203			
233814_at	50.578884	EFNA5	1946	Ephrin-A5
233871_at	42.2906			
233934_at	24.12915	LOC349160	349160	Hypothetical protein LOC349160
234030_at	36.926117			
234980_at	21.773212	TMEM56	148534	Transmembrane protein 56
235306_at	22.31179	GIMAP8	155038	GTPase, IMAP family member 8
236220_at	39.285076			
236365_at	38.961994			
236698_at	21.145327			
236740_at	21.301474			
237150_at	21.254215			
237427_at	20.181032			
237905_at	23.934755	KRT25	147183	Keratin 25
238009_at	20.990005			
238751_at	38.94119			
239443_at	26.224146	PCDHB6	56130	Protocadherin β 6
239447_at	44.547733	TRA2B	6434	Transformer 2 β homolog (<i>Drosophila</i>)
239477_at	24.663624	EFHB	151651	EF-hand domain family, member B
239698_at	24.539743			
240120_at	20.922508			
240214_at	31.374973			
240466_at	24.025442			
240964_at	21.602964			
241829_at	27.049297	FAM124A	220108	Family with sequence similarity 124A
241926_s_at	51.05444	ERG	2078	v-ets erythroblastosis virus E26 oncogene homolog (avian)
242003_at	20.770477	ERICH1	157697	Glutamate-rich 1
242249_at	41.35373			
242340_at	33.86683			
242721_at	46.80031			
242893_at	42.70509			
243500_at	30.1103	CASD1	64921	CAS1 domain containing 1
243746_at	45.71318	IGHMBP2	3508	Immunoglobulin mu binding protein 2
243901_at	28.134262	ALDOB	229	Aldolase B, fructose-bisphosphate
244531_at	61.498493	NNT	23530	Nicotinamide nucleotide transhydrogenase
1552835_at	21.108583	DENND1B	163486	DENN/MADD domain containing 1B
1552927_at	22.232943	MAP3K7IP3	257397	Mitogen-activated protein kinase kinase kinase 7 interacting protein 3
1552999_a_at	27.387323	WFDC10B	280664	WAP four-disulfide core domain 10B

Table II. Continued.

Probe set ID	Fold change	Gene symbol	Entrez gene	Gene title
1554176_a_at	23.11273	C3 or f33	285315	Chromosome 3 open reading frame 33
1554001_at	41.838184	TRIM37	4591	Tripartite motif-containing 37
1554398_at	24.453304	LYG2	254773	Lysozyme G-like 2
1555912_at	44.85555	ST7OT1	93653	ST7 overlapping transcript 1 (non-protein coding)
1556008_a_at	47.270073			
1556203_a_at	30.X93089	SRGAP2	23380	SLIT-ROBO Rho GTPase activating protein 2
1556289_at	20.807093			
1556357_s_at	26.665522	ERICH1	157697	Glutamate-rich 1
1556392_a_at	22.948168			
1556393_at	36.13576			
1557520_a_at	24.872932			
1558135_at	25.871822	TAF11	6882	TAF11 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 28 kDa
1558234_at	55.475685	FLJ36644	400617	Hypothetical protein LOC400617
1558574_at	26.385082			
1558606_s_at	20.5551			
1558693_s_at	29.909445	C1 or f85	112770	Chromosome 1 open reading frame 85
1558815_at	49.138927	SORBS2	8470	Sorbin and SH3 domain containing 2
1558934_a_at	37.3662	GTF2H5	404672	General transcription factor IIH, polypeptide 5
1560048_at	32.75761			
1560422_at	45.097427			
1561432_at	23.945683			
1561882_at	42.562286	SYTL3	94120	Synaptotagmin-like 3
1562094_at	28.180384			
1562294_x_at	22.353355	ANKRD30B	374860	Ankyrin repeat domain 30B
1562310_at	22.610895			
1562472_at	26.148716			
1562742_at	59.214592			
1562860_at	21.426584			
1563112_at	76.9244	FAM120AOS	158293	Family with sequence similarity 120A opposite strand
1564039_at	24.121265	ZSCAN23	222696	Zinc finger and SCAN domain containing 23
1568012_at	31.55809	CLIP1	6249	CAP-GLY domain containing linker protein 1
1570600_at	22.040146			

176 probe sets demonstrated a clear distinction between colon carcinoma lymphatic metastasis and hepatic metastasis, with the majority of probe sets (114 of 176) being downregulated in the hepatic metastasis and only a very small number being upregulated (62 of 176) (Table III).

Discussion

The purpose of this study was to investigate the heterogeneity in primary colon carcinoma and its corresponding lymphatic and hepatic metastases using GeneChip HGU133Plus2.0 expression arrays (Affymetrix). Our results demonstrate that the genome-wide gene expression varies between primary colon carcinoma and its paired lymphatic and hepatic metastases. In our study, we firstly demonstrate that a high degree of heterogeneity in gene expression exists in primary colon carcinoma and corresponding lymphatic and hepatic metastases.

The main purpose of investigating the heterogeneity found in primary tumors and corresponding metastases is to evaluate the effect of such heterogeneity on the efficacy of anticancer therapy and cancer patient prognosis. As previously discussed (1,16,18), the patient-derived tumor tissue (PDTT) xenograft model, which has a sound establishing method and a

retained similarity to the corresponding original donor tumors in histological presentation and biological behavior, including protein expression, tumor biomarker status and genomic and genetic status, has the potential to be an ideal strategy to achieve our purpose. In our previous study, we successfully established PDTT xenograft models of colon carcinoma with lymphatic and hepatic metastases using the same tissue samples from three above-mentioned tumor sites (16). The ideal biological characteristics of such PDTT xenograft models, as previously described (16), led to the consideration that such PDTT models may aid in the investigation of the underlying mechanism of heterogeneity-related anticancer therapy response differences in primary colon carcinoma and corresponding lymphatic and hepatic metastases.

In conclusion, in this study, we investigated the heterogeneity in primary colon carcinoma and corresponding lymphatic and hepatic metastases focusing on genome-wide gene expression, and revealed that there were differences in primary colon carcinoma and matched lymphatic and hepatic metastases. In subsequent studies, further investigation is required to ascertain the heterogeneity of functional genes found in primary colon carcinoma and paired lymphatic and hepatic metastases in a larger group of patients with metastatic

Table III. Genes differentially expressed in the colon carcinoma lymphatic metastasis specimen and the hepatic metastasis specimen.

Probe set ID	Fold change	Gene symbol	Entrez gene	Gene title
203323_at	17.572113	CAV2	858	Caveolin 2
203324_s_at	12.488382	CAV2	858	Caveolin 2
203372_s_at	38.86711	SOCS2	8835	Suppressor of cytokine signaling 2
203543_s_at	11.408775	KLF9	687	Kruppel-like factor 9
203561_at	28.196625	FCGR2A	2212	Fc fragment of IgG, low affinity IIa, receptor (CD32)
203835_at	11.724807	LRRC32	2615	Leucine-rich repeat containing 32
204035_at	22.833523	SCG2	7857	Secretogranin II (chromogranin C)
204069_at	18.195318	MEIS1	4211	Meis homeobox 1
204682_at	12.34678	LTBP2	4053	Latent transforming growth factor β binding protein 2
204802_at	34.692444	RRAD	6236	Ras-related associated with diabetes
205610_at	55.83588	MYOM1	8736	Myomesin 1, 185 kDa
205692_s_at	10.167213	CD38	952	CD38 molecule
205943_at	17.091461	TDO2	6999	Tryptophan 2,3-dioxygenase
206022_at	31.875471	NDP	4693	Norrie disease (pseudoglioma)
206025_s_at	11.98506	TNFAIP6	7130	Tumor necrosis factor, α -induced protein 6
206143_at	17.525316	SLC26A3	1811	Solute carrier family 26, member 3
206488_s_at	21.102892	CD36	948	CD36 molecule (thrombospondin receptor)
206654_s_at	10.075909	POLR3G	10622	Polymerase (RNA) III (DNA directed) polypeptide G (32 kD)
207089_at	34.273483	NRAP	4892	Nebulin-related anchoring protein
207501_s_at	19.537613	FGF12	2257	Fibroblast growth factor 12
207580_at	22.841743	MAGEB4	4115	Melanoma antigen family B, 4
208086_s_at	25.490797	DMD	1756	Dystrophin
208259_x_at	18.155922	IFNA7	3444	Interferon, α 7
209576_at	32.88002	GNAI1	2770	Guanine nucleotide binding protein (G protein), α inhibiting activity polypeptide 1
209652_s_at	12.313976	PGF	5228	Placental growth factor
209700_x_at	25.640623	PDE4DIP	9659	Phosphodiesterase 4D interacting protein
209776_s_at	13.3636875	SLC19A1	6573	Solute carrier family 19 (folate transporter), member 1
209821_at	20.940786	IL33	90865	Interleukin 33
210077_s_at	24.33262	SFRS5	6430	Splicing factor, arginine/serine-rich 5
210948_s_at	21.301382	LEF1	51176	Lymphoid enhancer-binding factor 1
211190_x_at	22.055872	CD84	8832	CD84 molecule
213423_x_at	10.081812	TUSC3	7991	Tumor suppressor candidate 3
213428_s_at	13.616949	COL6A1	1291	Collagen, type VI, α 1
214998_at	31.85507	AAK1	22848	AP2 associated kinase 1
215495_s_at	14.478629	SAMD4A	23034	Sterile α motif domain containing 4A
215513_at	17.604755	HYMAI	57061	Hydatidiform mole associated and imprinted (non-protein coding)
215657_at	12.403633	SLC26A3	1811	Solute carrier family 26, member 3
216466_at	13.427488	NAV3	89795	Neuron navigator 3
216589_at	10.48587	LOC390998	390998	Similar to hCG1644589
216695_s_at	18.244976	TNKS	8658	Tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase
216757_at	10.563152			
217157_x_at	15.075894	IGK	3514	Immunoglobulin κ locus
		IGKC	50802	Immunoglobulin κ constant
		LOC647506	647506	Similar to Ig κ chain V-I region HK101 precursor
		LOC650405	650405	Similar to Ig κ chain V-I region HK102 precursor
		LOC652493	652493	Similar to Ig κ chain V-I region HK102 precursor
217321_x_at	28.0056	ATXN3	4287	Ataxin 3
217665_at	12.379506			
218638_s_at	17.851858	SPON2	10417	Spondin 2, extracellular matrix protein
220858_at	19.423872	SORBS2	8470	Sorbin and SH3 domain containing 2
220906_at	20.039635			
221154_at	38.946198	TRIM49	57093	Tripartite motif-containing 49
221541_at	11.172807	CRISPLD2	83716	Cysteine-rich secretory protein LCCL domain containing 2
221558_s_at	10.16347	LEF1	51176	Lymphoid enhancer-binding factor 1
31835_at	11.370078	HRG	3273	Histidine-rich glycoprotein
223599_at	12.111252	TRIM6	117854	Tripartite motif-containing 6
223812_at	13.24775	FAM178B	51252	Family with sequence similarity 178, member B
223876_at	11.548148	SPATA16	83893	Spermatogenesis associated 16
224024_at	22.182268	ERGIC1	57222	Endoplasmic reticulum-golgi intermediate compartment (ERGIC) 1
224056_at	17.563913			

Table III. Continued.

Probe set ID	Fold change	Gene symbol	Entrez gene	Gene title
225029_at	15.641858	LOC550643	550643	Hypothetical LOC550643
225288_at	11.822223	COL27A1	85301	Collagen, type XXVII, α 1
226147_s_at	150.0156	PIGR	5284	Polymeric immunoglobulin receptor
226828_s_at	29.641703	HEYL	26508	Hairy/enhancer-of-split related with YRPW motif-like
227692_at	20.724081	GNAI1	2770	Guanine nucleotide binding protein (G protein), α inhibiting activity polypeptide 1
228167_at	34.570217	KLHL6	89857	Kelch-like 6 (<i>Drosophila</i>)
228275_at	21.79044			
228547_at	23.981333	NRXN1	9378	Neurexin 1
229051_at	26.098585			
229085_at	12.635592	LRRC3B	116135	Leucine-rich repeat containing 3B
229199_at	10.150482	SCN9A	6335	Sodium channel, voltage-gated, type IX, α subunit
229228_at	52.54796	CREB5	9586	cAMP responsive element binding protein 5
229381_at	11.871673	C1 or f64	149563	Chromosome 1 open reading frame 64
229518_at	28.762573	FAM46B	115572	Family with sequence similarity 46, member B
229659_s_at	20.485302			
229725_at	29.211315	ACSL6	23305	Acyl-CoA synthetase long-chain family member 6
229968_at	13.312329			
230261_at	16.136084	ST8SIA4	7903	ST8 α -N-acetyl-neuraminide α -2,8-sialyltransferase 4
230351_at	10.333499	LOC283481	283481	Hypothetical protein LOC283481
230615_at	19.222267	DUOXA2	405753	Dual oxidase maturation factor 2
230781_at	28.851234			
230943_at	13.1987	SOX17	64321	SRY (sex determining region Y)-box 17
231749_at	11.926773	TIMM13	26517	Translocase of inner mitochondrial membrane 13 homolog (yeast)
231964_at	27.403822			
232113_at	42.29946			
232378_at	14.133439	SLC5A9	200010	Solute carrier family 5 (sodium/glucose cotransporter), member 9
232408_at	11.252221	ZFYVE28	57732	Zinc finger, FYVE domain containing 28
232673_at	15.140463	LRRFIP2	9209	Leucine-rich repeat (in FLII) interacting protein 2
233586_s_at	23.207222	KLK12	43849	Kallikrein-related peptidase 12
233934_at	20.81878	LOC349160	349160	Hypothetical protein LOC349160
234233_s_at	15.17553			
235334_at	15.941539	ST6GALNAC3	256435	ST6 (α -N-acetyl-neuraminyl-2,3- β -galactosyl-1,3)-N-acetylglactosaminide α -2,6-sialyltransferase 3
235764_at	17.735975			
236105_at	11.190036			
236191_at	34.859856			
236762_at	13.769478	ATP9B	374868	ATPase, class II, type 9B
236801_at	14.398608			
237452_at	13.013656			
237608_at	11.315111			
238281_at	17.088953			
238283_at	11.590735	LOC151658	151658	Hypothetical LOC151658
238625_at	27.370302	C1orf168	199920	Chromosome 1 open reading frame 168
238634_x_at	12.341533			
238876_at	14.355665			
239506_s_at	30.187277	LOC151300	151300	Hypothetical LOC151300
239823_at	10.645902			
239883_s_at	47.341763	ANO4	121601	Anoctamin 4
239995_at	20.010996			
240212_at	19.915565			
240334_at	11.40588			
240856_at	22.449312			
241308_at	10.37427	LOC729589	729589	Hypothetical LOC729589
241765_at	33.79925	CPM	1368	Carboxypeptidase M
241841_at	16.08523			
242006_at	12.057802	LCA5	167691	Leber congenital amaurosis 5
242034_at	37.776375	FBXL17	64839	F-box and leucine-rich repeat protein 17
242193_at	10.155277	LOC100130155	100130155	Hypothetical protein LOC100130155
242246_x_at	17.554796			
242316_at	27.534788			
242509_at	21.54953			

Table III. Continued.

Probe set ID	Fold change	Gene symbol	Entrez gene	Gene title
242906_at	27.747772			
242943_at	14.356349	ST8SIA4	7903	ST8 α -N-acetyl-neuraminide α -2,8-sialyltransferase 4
243489_at	14.7732115			
244009_at	10.311149			
244074_at	12.198139	LOC100129104	100129104	Similar to hydroxyproline-rich glycoprotein VSP-3
244112_x_at	17.781418			
1552440_at	22.730635	GPR182	11318	G protein-coupled receptor 182
1552463_at	17.636255	SERPINB11	89778	Serpin peptidase inhibitor, clade B (ovalbumin), member 11 (gene/pseudogene)
1552584_at	11.233118	IL12RB1	3594	Interleukin 12 receptor, β 1
1553470_at	12.427377	DNAH17	8632	Dynein, axonemal, heavy chain 17
1553626_a_at	15.016963	C17orf57	124989	Chromosome 17 open reading frame 57
1553970_s_at	10.47964	CEL	1056	Carboxyl ester lipase (bile salt-stimulated lipase)
1554037_a_at	11.989659	ZBTB24	9841	Zinc finger and BTB domain containing 24
1554277_s_at	10.488272	FANCM	57697	Fanconi anemia, complementation group M
1555745_a_at	11.363364	LYZ	4069	Lysozyme (renal amyloidosis)
1554672_at	19.961636	TTC26	79989	Tetratricopeptide repeat domain 26
1555002_at	22.022196	MGC39545	403312	Hypothetical protein LOC403312
1555022_at	25.609352	RGS12	6002	Regulator of G-protein signaling 12
1555339_at	12.859835	RAP1A	5906	RAP1A, member of RAS oncogene family
1555435_at	36.40531	AFF4	27125	AF4/FMR2 family, member 4
1556474_a_at	52.13133	FLJ38379	285097	Hypothetical FLJ38379
1556877_at	25.908909			
1556898_at	11.959699	LOC200830	200830	Hypothetical protein LOC200830
1556905_at	32.662815	ZNF577	84765	Zinc finger protein 577
1557581_x_at	10.408871			
1557753_at	14.301804			
1558452_at	10.139205	TMEM144	55314	Transmembrane protein 144
1558706_a_at	18.524925	ATOH8	84913	Atonal homolog 8 (<i>Drosophila</i>)
1558999_x_at	17.685259	LOC283922	283922	Pyruvate dehydrogenase phosphatase regulatory subunit pseudogene
		PDPR	55066	Pyruvate dehydrogenase phosphatase regulatory subunit
1559057_at	13.380709			
1559101_at	11.838457	FYN	2534	FYN oncogene related to SRC, FGR, YES
1560756_at	22.905785			
1560996_at	22.832338			
1560999_a_at	38.31254			
1561521_at	27.853552	S100B	6285	S100 calcium binding protein B
1561882_at	37.053608	SYTL3	94120	Synaptotagmin-like 3
1562094_at	12.56786			
1562165_at	10.340052			
1562367_at	17.341137	C15 or f54	400360	Chromosome 15 open reading frame 54
1562528_at	24.569925			
1562716_at	11.445873	LOC284632	284632	Hypothetical LOC284632
1562990_at	15.226481			
1563061_at	30.11877			
1563077_at	14.259786			
1563612_at	25.13671			
1563827_at	13.029244	LOC158434	158434	Hypothetical protein LOC158434
1564003_at	17.397495			
1564294_at	12.534436			
1564299_at	25.326767	LOC100127940	100127940	Hypothetical LOC100127940
1564386_at	36.96616	TXNDC8	255220	Thioredoxin domain containing 8 (spermatozoa)
1564653_s_at	17.913086	LEKR1	389170	Leucine, glutamate and lysine rich 1
1565668_at	15.500862	TUBA1B	10376	Tubulin, α 1b
1565755_at	22.79346	MAP1D	254042	Methionine aminopeptidase 1D
1566767_at	12.185921			
1567702_at	19.35184			
1568696_at	24.071138	ARMETL1	441549	Arginine-rich, mutated in early stage tumors-like 1
1569218_at	40.51703			
1569408_at	26.201654	EIF2C4	192670	Eukaryotic translation initiation factor 2C, 4
1569788_at	15.160305	ST8SIA1	6489	ST8 α -N-acetyl-neuraminide α -2,8-sialyltransferase 1
1570486_at	12.096198	SOX6	55553	SRY (sex determining region Y)-box 6

colon carcinoma, in order to identify specific functional genes related to lymphatic and hepatic metastases.

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