Identification of differentially expressed genes in human bladder cancer through genome-wide gene expression profiling

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Abstract. Large-scale gene expression profiling is an effective strategy for understanding the progression of bladder cancer (BC). The aim of this study was to identify genes that are expressed differently in the course of BC progression and to establish new biomarkers for BC. Specimens from 21 patients with pathologically confirmed superficial (n=10) or invasive (n=11) BC and 4 normal bladder samples were studied; samples from 14 of the 21 BC samples were subjected to microarray analysis. The validity of the microarray results was verified by real-time RT-PCR. Of the 136 up-regulated genes we detected, 21 were present in all 14 BCs examined (100%), 44 in 13 (92.9%), and the other 71 in 12 BCs (85.7%). Of 69 down-regulated genes, 25 were found in all 14 BCs (100%), 22 in 13 (92.9%), and the other 22 in 12 BCs (85.7%). Functional annotation revealed that of the up-regulated genes, 36% were involved in metabolism and 14% in transcription and processing; 25% of the down-regulated genes were linked to cell adhesion/surface and 21% to cytoskeleton/cell membrane. Real-time RT-PCR confirmed the microarray results obtained for the 6 most highly up- and the 2 most highly down-regulated genes. Among the 6 most highly upregulated genes, CKS2 was the only gene with a significantly greater level of up-regulation in invasive than in superficial BC (p=0.04). To confirm this result, we subjected all 21 BC samples to real-time PCR assay for CKS2. We found a considerable difference between superficial and invasive BC (p=0.001). Interestingly, there was a considerable difference between the normal bladder and invasive BC (p=0.001) and less difference between the normal bladder and superficial BC (p=0.005). We identified several genes as promising candidates for diagnostic biomarkers of human BC and the

Key words: microarray, expression profile, bladder cancer, CKS2

CKS2 gene not only as a potential biomarker for diagnosing, but also for staging human BC. This is the first report demonstrating that *CKS2* expression is strongly correlated with the progression of human BC.

Introduction

Bladder cancer (BC) is among the 5 most common malignancies worldwide, and the 2nd most common tumor of the genitourinary tract and the 2nd most common cause of death in patients with cancer of the urinary tract (1-7). At diagnosis, BC with progression usually appears to be superficial (pT1); 20% of tumors with muscle invasion at the time of diagnosis tend to progress rapidly and their prognosis is unfavorable (3-5). The ability to predict, at the first biopsy, whether a BC shift to progression is probable would facilitate the selection of appropriate treatment modalities and improve the prognosis of patients with this cancer. Due to their insufficient sensitivity and specificity, none of the biomarkers now available for the diagnosis of BC can replace cystoscopy or cytology (3,6,7) and patients with suspected BC continue to be subjected to painful cystoscopy.

Gene expression profiling has been used in the molecular classification of many tumor types. Molecular subtypes with potential diagnostic and prognostic implications have been identified (3,8-10). DNA microarrays aid in the outcome prediction for cancer patients because they facilitate the simultaneous analysis of the expression profiles of thousands of genes, making possible the identification of groups of genes with different expression profiles in tumors related to different outcomes. These gene-expression profiles assist in the selection of optimum treatment strategies by allowing therapies to be precisely adapted to different types of tumors (8-10).

Microarray analysis have identified cancer-related genes in BC (3,4,11-15). For example, the p33 inhibitor of the growth family 1 (p33ING1) and cathepsin E (CTSE) expression level, are correlated with the progression and prognosis of BC (11,14). While these studies provided useful insights into the molecular biology of BC, their sensitivity and specificity are limited and their usefulness for

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Table I. Patient characteristics.

No.	Age	Gender	Stage	Grade
1	55	F	superficial	G2
2	61	Μ	superficial	G2
3	68	М	superficial	G2
4	68	М	superficial	G2
5	88	М	invasive	G3
6	80	М	invasive	G3
7	53	F	superficial	G2
8	85	М	superficial	G2
9	53	М	superficial	G2
10	78	М	invasive	G3
11	83	М	invasive	G2
12	74	F	superficial	G2
13	89	М	superficial	G3
14	70	М	invasive	G3
15	47	F	superficial	G2
16	71	М	invasive	G3
17	75	М	invasive	G3
18	87	F	invasive	G2
19	76	М	invasive	G3
20	59	М	invasive	G3
21	78	М	invasive	G2

predicting disease outcome remains unclear (13-15). Using gene expression analysis and DNA microarrays, we looked for novel gene clusters related to human BC in an attempt to discover new biomarkers.

Materials and methods

Tissue samples. Tissue samples were obtained from 21 patients with BC (10 superficial, 11 invasive) who had undergone cystectomy or transurethral resection of bladder tumors at Kagoshima University Hospital, Kagoshima, Japan (Table I). Each tumor was staged and graded according to the TNM staging system (16) and the Japanese Urological Association and the Japanese Society of Pathology (17). Our study was approved by the Bioethics Committee of Kagoshima University; written prior informed consent was obtained from all patients for use of their samples and clinical and pathological data.

Sample preparation and total RNA extraction. Freshly harvested tissues, immediately frozen in liquid nitrogen and stored at -80°C, were dissolved in TRIzol reagent (Invitrogen, Carlsbad, CA, USA); for total RNA extraction we followed the manufacturer's protocol. We used premium total RNA (from normal human bladder; Clontech, Palo Alto, CA, USA) as a reference for microarray analysis. RNA density was measured in an Ultrospec 3100 Pro instrument (Amersham Biosciences), RNA quality was checked in an Agilent 2100 bioanalyzer (Agilent Technologies).

Antisense RNA (aRNA) amplification. For microarray analysis we used good-quality total RNA samples from 9 patients with superficial- and 5 patients with invasive BC (samples 1-14, Table I). aRNA was amplified from 5 μ g total RNA using the amino allyl message Amp aRNA amplification kit (Ambion, Austin, TX, USA). We amplified single-strand cDNA using T7 oligos (dT), converted the product into double-stranded cDNA, purified this cDNA, and then performed amplification from double-strand cDNA templates using the manufacturer's protocol.

Dye coupling and microarray hybridization. Oligoarrays, AceGene[®] human oligo chip 30K (http://bio.hitachi-sk. co.jp/acegene/index.html, Hitachi Software Engineering Co. Ltd., Yokohama, Japan) spotted with ~30,144 genes, were used for dye coupling (normal bladder -Cy3, BC -Cy5) and microarray hybridization. Pellets were formed with ethanolprecipitated aRNA (5 μ g) and 5 μ l CyDye (Amersham Bioscience); 5x fragmentation buffer was added after purification and following further refinement, we obtained concentrated coupled aRNA. A hybridization solution was added to the microarrays and this was followed by 18-h incubation at 50°C.

cDNA preparation and quantitative real-time RT-PCR. Total RNA (2 μ g) was mixed with 0.5 μ g of oligo-dT primer and 0.4 μ l of dNTPs (25 mM); a final volume of 25 μ l was prepared for single-strand synthesis. Using the manufacturer's protocol, we prepared 21 cDNA samples from the same total RNA used for microarray analysis (n=14) and from 7 additional BCs. For normal bladder controls we prepared 4 cDNA samples from 3 different lots of premium total RNA (human normal bladder, Clontech) and human bladder total RNA (Chemicon International, Inc., Temecula, CA, USA). Gene-specific PCR products were assayed continuously using a 7300 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Briefly, the initial PCR step was a 10 min hold at 95°C; the cycles (n=40) consisted of a 15-sec denaturation step at 95°C followed by 1-min annealing/extension at 63°C. Primers used for real-time PCR were as follows: FABP5: 5'ttggttcagcatcaggagtg-3' (sense), 5'-cctgtccaaagtgatg atgg-3' (antisense); PABPC1: 5'-agccatgcaccctactctg-3' (sense), 5'tctttagcttggtgggcttg-3' (antisense); CKS2: 5'-catgagccagaa ccacatattc-3' (sense), 5'-cagctcatgcacaggtatgg-3' (antisense); SF3B1: 5'-ccctagagggcctgagagtt-3' (sense), 5'-tgtgctatgagagc gtcctg-3' (antisense); DDX5: 5'-tcccaagttgcttcagttgg-3' (sense), 5'-ccttttgcccgcagagtatc-3' (antisense); EIF3S6: 5'cttggtggcttgtcttgagg-3' (sense), 5'-atcttggcatccagtcttgc-3' (antisense); TAGLN: 5'-aagaatgatgggcactaccg-3' (sense), 5'actgatgatctgccgaggtc-3' (antisense); TPM2: 5'-actgga caacgcactcaatg-3' (sense), 5'-gttggcaatttctgctcctc-3' (antisense); RPL37A: 5'-taccaagaaagtcgggatcg-3' (sense), 5'tcttcatgcaggaaccacag-3' (antisense). All reactions were performed in duplicate and a negative control lacking cDNA was included. The gene encoding ribosomal protein L37a (RPL37A) served as an internal control because in our hands it showed the smallest Cy5/Cy3 fluctuation. RPL37A located on chromosome 2q35q is quite different from RPL37 located on chromosome 5p13 (Table II). The relative mRNA expression examined was normalized to the amount of RPL37A in the same cDNA using the standard curve method provided by the manufacturer.

Table II. Frequently up-regulated genes in bladder cancer.

No.	Symbol	Gene name	Accession	UniGene	Location	Median	% Up	Function
1	FABP5	fatty acid binding protein 5 (psoriasis-associated)	NM_001444	Hs.408061	8q21.13	5.873	100.0%	metabolism
2	PABPC1	poly(a) binding protein, cytoplasmic 1	NM_002568	Hs.387804	8q22.2-q23	4.512	100.0%	metabolism
3	CKS2	cdc28 protein kinase 2	BC006458	Hs.83758	9q22	4.500	100.0%	cell proliferatio
4	SF3B1	splicing factor 3b, subunit 1, 155kd	NM_012433	Hs.567433	2q33.1	4.086	100.0%	translation and processing
5	DDX5	dead/h (asp-glu-ala-asp/his) box polypeptide 5	NM_004396	Hs.279806	17q21	3.801	100.0%	metabolism
6	DJ465N24.2.1	hypothetical protein dj465n24.2.1	NM_020317	Hs.259412	1p36.13-p35.1	3.764	100.0%	EST
7	EIF3S6	murine mammary tumor integration site 6	NM_001568	Hs.405590	8q22-q23	3.733	100.0%	translation and processing
8	PSMA4	proteasome (prosome, macropain) subunit, alpha type, 4	NM_002789	Hs.251531	15q25.1	3.685	100.0%	metabolism
9	SHFM1	deleted in split-hand/split-foot 1 region	NM_006304	Hs.489201	7q21.3-q22.1	3.617	100.0%	metabolism
10	HSPE1	heat shock 10kd protein 1 (chaperonin 10)	NM_002157	Hs.1197	2q33.1	3.559	100.0%	metabolism
11	PSMA3	proteasome (prosome, macropain) subunit, alpha type, 3	NM_002788	Hs.531089	14q23	3.522	100.0%	metabolism
12	RPS24	ribosomal protein s24, isoform c	NM_001026	Hs.356794	10q22-q23	3.392	100.0%	translation
								and processing
13	NPM1	nuclear phosphoprotein b23 clone hpb2	M31004	Hs.557550	5q35	3.281	100.0%	transcription
								and processing
14	SFRS7	splicing factor, arginine/ serine-rich 7 (35kd)	-	Hs.309090	2p22.1	3.266	100.0%	transcription
								and processing
15	CCT2	chaperonin containing tcp1, subunit 2 (beta)	NM_006431	Hs.189772	12q15	3.006	100.0%	cell proliferation
16	PFDN4	prefoldin 4	BC010953	Hs.91161	20q13.2	2.914	100.0%	metabolism
17	HNRPA2B1	heterogeneous nuclear	NM_031243	Hs.487774	7p15	2.634	100.0%	transcription
		ribonucleoprotein a2/b1, isoform b1						and processing
18	MIF	macrophage migration inhibitory factor	NM_002415	Hs.407995	22q11.23	2.585	100.0%	metabolism
19	RPA3	replication protein a3 (14kd)	NM_002947	Hs.487540	7p22	2.447	100.0%	others
20	UQCRH	mitochondrial hinge protein precursor	M36647	Hs.481571	1p34.1	2.409	100.0%	mitochondrion
21	RPL37	ribosomal protein 137	NM_000997	Hs.80545	5p13	2.328	100.0%	translation
	111 207		1111_0000000	110100010	opro	21020	1001070	and processing
22	KRT18	keratin 18	NM_000224	Hs.406013	12q13	7.996	92.9%	cytoskeleton/ cell membrane
23	KRT7	keratin 7	NM_005556	Hs.411501	12q12-q13	6.931	92.9%	cytoskeleton/ cell membrane
24	KRT19	keratin 19	NM_002276	Hs.514167	17q21.2	6.133	92.9%	cytoskeleton/ cell membrane
25	HSPCA	heat shock 90kd protein 1, alpha	NM_005348	Hs.525600	14q32.33	6.004	92.9%	metabolism
25 26	KRT8	keratin 8	NM_002273	Hs.533782	14q52.55 12q13	5.206	92.9%	cytoskeleton/
								cell membrane
27	DECR1	2,4-dienoyl coa reductase 1 precursor	NM_001359	Hs.492212	8q21.3	4.714	92.9%	metabolism
28	SH3YL1	SH3 domain containing, Ysc84-like 1	NM_015677	Hs.515951	2p25.3	4.334	92.9%	others
29	TACSTD1	tumor-associated calcium signal transducer 1 precursor	NM_002354	Hs.692	2p21	4.306	92.9%	cytoskeleton/ cell membrane
30	CCT5	chaperonin containing TCP1, subunit 5 (epsilon)	BC002971	Hs.1600	5p15.2	4.291	92.9%	metabolism
31	HMGN1	High-mobility group nucleosome binding domain 1	AK056033	Hs.356285	21q22.3	3.819	92.9%	transcription and processing
20	TXNL5	thioredoxin-like 5	BC006405	Ha 408226	17-12-1	3.761	92.9%	metabolism
32 33	PSMA5		NM_002790	Hs.408236 Hs.485246	17p13.1	3.623	92.9% 92.9%	metabolism
		proteasome (prosome, macropain) subunit, alpha type, 5	_		1p13			
34	TXN	thioredoxin	NM_003329	Hs.435136	9q31	3.426	92.9%	metabolism
35	LDHB	lactate dehydrogenase B	NM_002300	Hs.446149	12p12.2-p12.1	3.357	92.9%	metabolism
36	SNRPD1	small nuclear ribonucleoprotein d1 polypeptide (16kd)	NM_006938	Hs.464734	18q11.2	3.354	92.9%	transcription and processing
37	MARCKS	myristoylated alanine-rich protein kinase C substrate	NM_002356	Hs.519909	6q22.2	3.224	92.9%	others
38	NME1	non-metastatic cells 1 protein	NM_000269	Hs.118638	17q21.3	3.209	92.9%	metabolism
39	EIF2S2	eukaryotic translation initiation factor 2, subunit 2 (beta, 38kd)	NM_003908	Hs.429180	20pter-q12	3.194	92.9%	translation and processing
		Subulifi 2 (beta, Soku)						and processing

Table II. Continued.

41CPNE3 PCNA BALcopine iii proliferating cell nuclear antigen proliferating cell nuclear antigen ebna1 binding protein 2NM_003099 NM_002592 NM_006824Hs.191219 Hs.147433 BAL47433 BAL47433 20pter-p12 3.062 3.03043 <i>EBNA1BP2</i> ebna1 binding protein 2NM_002592 NM_006824Hs.147433 Hs.346868 $1p35-p33$ 3.03044 <i>HSPC016</i> guanine nucleotide binding protein-like 3 nuclear factor (erythroid-derived 2)-like 2NM_014366 NM_006164Hs.313544 Hs.135544 $3p21.1$ 2.891 2.87646 <i>NFE2L2</i> nuclear factor (erythroid-derived 2)-like 2NM_006164 NM_000164Hs.155396 Hs.1255396 $2q31$ 2.87647 <i>XEDAR</i> ribosomal protein 16NM_0121783 NM_000970Hs.491912 Hs.42623 $8q13.2$ 2.781 homolog) subunit 550 <i>ALK</i> anaplastic lymphoma kinase (Ki-1)NM_006837 NM_004304Hs.196534 Hs.1912 $2p23$ 2.76551 <i>STATIP1</i> signal transducer and activator of transcription 3 interacting protein 1NM_004304 NM_004889Hs.125113 Hs.125113 21q22.11 2.572 2.572 2.524 complex, subunit f, isoform 254 <i>ATP571</i> atp synthase, h+ transporting, mitochondrial f0 complex, subunit b, isoform 1 prospherylycerate kinase 1 NM_002291NM_00291 Hs.78771 Hs.4395481p13.2 2.519 2.5192.519 2.51955 <i>PGK1</i> phosphoglycerate kinase 1 swi/shr related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4NM_003072 Hs.327527Hs.327527 19p13.22.411 2.421	92.9% 92.9% 92.9% 92.9% 92.9% 92.9% 92.9% 92.9% 92.9% 92.9%	metabolism cell proliferation translation and processing EST cell proliferation transcription and processing others signal transduction transcription
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47XEDAR RPL6x-linked ectodysplasin receptor ribosomal protein 16NM_021783 NM_000970Hs.302017 Hs.546283Xq12 12q24.12.806 2.79049COPS5cop9 (constitutive photomorphogenic, arabidopsis, homolog) subunit 5NM_006837Hs.491912 $8q13.2$ 2.78150ALKanaplastic lymphoma kinase (Ki-1)NM_004304Hs.196534 $2p23$ 2.76551STATIP1signal transducer and activator of transcription 3 interacting protein 1NM_004304Hs.196534 $2p23$ 2.61152CC78chaperonin containing tep1, subunit 8 (theta)NM_006585Hs.125113 $21q22.11$ 2.57253ATP5J2atp synthase, h+ transporting, mitochondrial f0 complex, subunit f, isoform 2NM_001688Hs.514870Ip13.22.51954ATP5F1atp synthase, h+ transporting, mitochondrial f0 complex, subunit b, isoform 1NM_002291Hs.78771Xq132.50355PGK1 Phosphoglycerate kinase 1NM_002291Hs.78271Xq132.50356FL/22875 Hypothetical protein FL/22875NM_032231Hs.43954815q22.312.43157LPIN3Homo sapiens lipin 3NM_003072Hs.32752719p13.22.411	92.9% 92.9% 92.9% 92.9%	and processing others signal transduction transcription
48RPL6ribosomal protein 16NM_000970Hs.54628312q24.12.79049COPS5cop9 (constitutive photomorphogenic, arabidopsis, homolog) subunit 5NM_006837Hs.4919128q13.22.78150ALKanaplastic lymphoma kinase (Ki-1)NM_004304Hs.1965342p232.76551STATIP1signal transducer and activator of transcription 3 interacting protein 1NM_018255Hs.873918q12.22.61152CC78chaperonin containing tcp1, subunit 8 (theta)NM_006585Hs.12511321q22.112.57253ATP5J2atp synthase, h+ transporting, mitochondrial f0 complex, subunit f, isoform 2NM_001688Hs.5148701p13.22.51954ATP5F1atp synthase, h+ transporting, mitochondrial f0 complex, subunit b, isoform 1NM_00291Hs.78771Xq132.50355PGK1 Phosphoglycerate kinase 1NM_002291Hs.78771Xq132.50356FLJ22875 Hypothetical protein FLJ22875NM_032231Hs.43954815q22.312.43157LPIN3Homo sapiens lipin 3NM_003072Hs.32752719p13.22.411	92.9% 92.9% 92.9%	signal transduction transcription
49 $COPS5$ $cop9$ (constitutive photomorphogenic, arabidopsis, homolog) subunit 5NM_006837Hs.491912 $8q13.2$ 2.781 50 ALK anaplastic lymphoma kinase (Ki-1)NM_004304Hs.196534 $2p23$ 2.765 51 $STATIP1$ signal transducer and activator of transcription 3 interacting protein 1NM_018255Hs.8739 $18q12.2$ 2.611 52 $CCT8$ chaperonin containing tcp1, subunit 8 (theta)NM_006585Hs.125113 $21q22.11$ 2.572 53 $ATP5J2$ atp synthase, h+ transporting, mitochondrial f0 complex, subunit f, isoform 2NM_001688Hs.514870 $1p13.2$ 2.519 54 $ATP5F1$ atp synthase, h+ transporting, mitochondrial f0 complex, subunit b, isoform 1NM_000291Hs.78771Xq13 2.503 55 $PGK1$ phosphoglycerate kinase 1NM_002231Hs.439548 $15q22.31$ 2.431 56 $FLJ22875$ hypothetical protein FLJ22875NM_022296Hs.528618 $20q11.2-q12$ 2.428 58 $SMARCA4$ swi/snf related, matrix associated, actin dependentNM_003072Hs.327527 $19p13.2$ 2.411	92.9% 92.9%	transduction transcription
111111111homolog) subunit 550ALKanaplastic lymphoma kinase (Ki-1)NM_004304Hs.196534 $2p23$ 2.76551STATIP1signal transducer and activator of transcription 3 interacting protein 1NM_018255Hs.873918q12.22.61152CCT8chaperonin containing tcp1, subunit 8 (theta)NM_006585Hs.12511321q22.112.57253ATP5J2atp synthase, h+ transporting, mitochondrial f0 complex, subunit f, isoform 2NM_001688Hs.5210567q22.12.52454ATP5F1atp synthase, h+ transporting, mitochondrial f0 complex, subunit b, isoform 1NM_001688Hs.78771Xq132.50355PGK1phosphoglycerate kinase 1NM_002291Hs.78771Xq132.50356FLJ22875hypothetical protein FLJ22875NM_032231Hs.43954815q22.312.43157LPIN3Homo sapiens lipin 3NM_003072Hs.32752719p13.22.411	92.9%	
51STATIP1signal transducer and activator of transcription 3 interacting protein 1NM_018255Hs.873918q12.22.61152CCT8chaperonin containing tcp1, subunit 8 (theta)NM_006585Hs.12511321q22.112.57253ATP5J2atp synthase, h+ transporting, mitochondrial f0 complex, subunit f, isoform 2NM_004889Hs.5210567q22.12.52454ATP5F1atp synthase, h+ transporting, mitochondrial f0 complex, subunit b, isoform 1NM_001688Hs.5148701p13.22.51955PGK1phosphoglycerate kinase 1NM_000291Hs.78771Xq132.50356FLJ22875hypothetical protein FLJ22875NM_032231Hs.43954815q22.312.43157LPIN3Homo sapiens lipin 3NM_0022896Hs.52861820q11.2-q122.42858SMARCA4swi/snf related, matrix associated, actin dependentNM_003072Hs.32752719p13.22.411		and processing
52 CC78 chaperonin containing tcp1, subunit 8 (theta) NM_006585 Hs.125113 21q22.11 2.572 53 ATP5J2 atp synthase, h+ transporting, mitochondrial f0 complex, subunit f, isoform 2 NM_004889 Hs.521056 7q22.1 2.524 54 ATP5F1 atp synthase, h+ transporting, mitochondrial f0 complex, subunit b, isoform 1 NM_001688 Hs.514870 1p13.2 2.519 55 PGK1 phosphoglycerate kinase 1 NM_000291 Hs.78771 Xq13 2.503 56 FLJ22875 hypothetical protein FLJ22875 NM_032231 Hs.439548 15q22.31 2.431 57 LPIN3 Homo sapiens lipin 3 NM_003072 Hs.327527 19p13.2 2.428	92.9%	cytoskeleton/ cell membrane
53ATP5J2atp synthase, h+ transporting, mitochondrial f0 complex, subunit f, isoform 2NM_004889Hs.5210567q22.12.52454ATP5F1atp synthase, h+ transporting, mitochondrial f0 complex, subunit b, isoform 1NM_001688Hs.5148701p13.22.51955PGK1phosphoglycerate kinase 1NM_000291Hs.78771Xq132.50356FLJ22875hypothetical protein FLJ22875NM_032231Hs.43954815q22.312.43157LPIN3Homo sapiens lipin 3NM_022896Hs.52861820q11.2-q122.42858SMARCA4swi/snf related, matrix associated, actin dependentNM_003072Hs.32752719p13.22.411		transcription and processing
54 ATP5F1 atp synthase, h+ transporting, mitochondrial f0 complex, subunit b, isoform 1 NM_001688 Hs.514870 1p13.2 2.519 55 PGK1 phosphoglycerate kinase 1 NM_000291 Hs.78771 Xq13 2.503 56 FLJ22875 hypothetical protein FLJ22875 NM_032231 Hs.439548 15q22.31 2.431 57 LPIN3 Homo sapiens lipin 3 NM_022896 Hs.528618 20q11.2-q12 2.428 58 SMARCA4 swi/snf related, matrix associated, actin dependent NM_003072 Hs.327527 19p13.2 2.411	92.9%	metabolism
State Complex, subunit b, isoform 1 55 PGK1 phosphoglycerate kinase 1 NM_000291 Hs.78771 Xq13 2.503 56 FLJ22875 hypothetical protein FLJ22875 NM_032231 Hs.439548 15q22.31 2.431 57 LPIN3 Homo sapiens lipin 3 NM_022896 Hs.528618 20q11.2-q12 2.428 58 SMARCA4 swi/snf related, matrix associated, actin dependent NM_003072 Hs.327527 19p13.2 2.411	92.9%	mitochondrion
56 FLJ22875 hypothetical protein FLJ22875 NM_032231 Hs.439548 15q22.31 2.431 57 LPIN3 Homo sapiens lipin 3 NM_022896 Hs.528618 20q11.2-q12 2.428 58 SMARCA4 swi/snf related, matrix associated, actin dependent NM_003072 Hs.327527 19p13.2 2.411	92.9%	mitochondrion
57 LPIN3 Homo sapiens lipin 3 NM_022896 Hs.528618 20q11.2-q12 2.428 58 SMARCA4 swi/snf related, matrix associated, actin dependent NM_003072 Hs.327527 19p13.2 2.411	92.9%	metabolism
57 LPIN3 Homo sapiens lipin 3 NM_022896 Hs.528618 20q11.2-q12 2.428 58 SMARCA4 swi/snf related, matrix associated, actin dependent NM_003072 Hs.327527 19p13.2 2.411	92.9%	EST
	92.9%	metabolism
	92.9%	transcription and processing
59 <i>PSMA2</i> proteasome (prosome, macropain) subunit, NM_002787 Hs.333786 7p14.1 2.397 alpha type, 2	92.9%	metabolism
60 MEOX2 mesenchyme homeo box 2 NM_005924 Hs.527007 7p22.1-p21.3 2.397	92.9%	transcription and processing
61 CACYBP calcyclin binding protein NM_014412 Hs.508524 1q24-q25 2.336	92.9%	metabolism
62 SNRPE small nuclear ribonucleoprotein polypeptide e NM_003094 Hs.334612 1q32 2.286	92.9%	transcription and processing
63 SFT2D1 SFT2 domain containing 1 NM_145169 Hs.487143 6q27 2.241	92.9%	cytoskeleton/ cell membrane
64 <i>TCEB1</i> transcription elongation factor B (SIII), NM_005648 Hs.554594 8q21.11 2.132 polypeptide 1	92.9%	transcription and processing
65CRHR2corticotropin releasing hormone receptor 2NM_001883Hs.5462467p14.32.101	92.9%	signal transduction
66 HSPA4 apg-2 AB023420 Hs.90093 5q31.1-q31.2 4.052	85.7%	metabolism
67 DNAJA1 heat shock protein, dnaj-like 2 NM_001539 Hs.445203 9p13-p12 4.003	85.7%	metabolism
68 CSTB cystatin b (stefin b) NM_000100 Hs.695 21q22.3 3.611	85.7%	others
69 <i>RPL26</i> ribosomal protein 126 NM_000987 Hs.528879 17p13 3.456	85.7%	metabolism
70CBX3heterochromatin-like protein 1BC000954Hs.3811897p15.23.419	85.7%	transcription and processing
71 GA17 dendritic cell protein; ga17 NM_006360 Hs.502244 11p13 3.417	85.7%	cytoskeleton/ cell membrane
72 \$100A2 \$100 calcium binding protein A2 NM_005978 Hs.516484 1q21 3.285	85.7%	others
73TAX1BP1tax1 (human t-cell leukemia virus type i)NM_006024Hs.345767p153.284binding protein 1	85.7%	cell proliferatior
74 CUL3 cullin 3 NM_003590 Hs.372286 2q36.3 3.272	85.7%	others
75 EPRS glutamyl-prolyl trna synthetase NM_004446 Hs.497788 1q41-q42 3.266	85.7%	metabolism
76 <i>S100A1</i> s100 calcium-binding protein a11 (calgizzarin) BC001410 Hs.417004 1q21 3.259	85.7%	metabolism
77HSPC064WD repeats and SOF1 domain containingNM_015420Hs.5322658q22.33.229	85.7%	others
78 CETN3 centrin 3 NM_004365 Hs.128073 5q14.3 3.141		metabolism
79 PDCD10 programmed cell death 10 NM_007217 Hs.478150 3q26.1 3.139	85.7%	
80 WWOX for ii protein AF227527 Hs.461453 16q23.3-q24.1 2.873		
81 TRIM33 tripartite motif-containing 33 protein NM_015906 Hs.26837 1p13.1 2.850	85.7% 85.7% 85.7%	others metabolism

Table II. Continued.

91 ATP2B1 atpase, ca ⁺⁺ transporting, plasma membrane 1 NM_001682 Hs.506276 12q21.3 2.690 85.7% metabolism 92 LOC554202 hypothetical LOC554202 DnaJ (Hsp40) homolog, subfamily C, member 7 NM_003315 Hs.458096 9p21.3 2.687 85.7% EST 93 DNAJC7 DnaJ (Hsp40) homolog, subfamily C, member 7 NM_003315 Hs.500156 17q11.2 2.687 85.7% metabolism 94 KIAA0220 KIAA0220-like protein XM_290670 Hs.531664 16p12.3 2.659 85.7% others 95 - ensembl genscan prediction AC026900 - chromosome 1 2.654 85.7% EST 96 NEK6 putative serine-threonine protein kinase NM_014397 Hs.197071 9q33.3-q34.11 2.616 85.7% metabolism 97 FIBL-6 weakly similar to fibulin-1 isoform d precursor AK027344 Hs.58877 1q25.3-q31.1 2.612 85.7% metabolism 98 RPS7 ribosomal protein s7 NM_001011 Hs.546287 2p25 2.588 85.7% EST	No.	Symbol	Gene name	Accession	UniGene	Location	Median	% Up	Function
84 CVCX Fordename NN_018447 14.37000 791.3 2.72 8.73 material 86 VXX biguin specific periodus 47 NM_01794 H5.5752 1191.53 2.706 8.73 methodium 87 RAB1A nb11.m.mether no oncogene family NM_06860 H5.5714 1521.2-923.1 2.08 8.74 methodium 88 PXMD14 26 protensome-associated pair loan of open reading fame 12 NM_00313 H4.69538 1541.2 2.09 8.75 methodiosin 90 R17 reinobascom 1.5 open reading fame 12 NM_00313 H4.69838 1541.2 2.09 8.75 cell profilemoli 91 AT72.B1 mayse, ct rumsporting, plana membane 1 NM_00313 H4.69838 1541.2 2.087 8.75 metabolism 92 AXA7C7 band (Hap/to) bonolog, ubfamily C. menther 1 NM_00301 H5.49806 1621.3 2.64 8.75 metabolism 94 ALAO220 Iscomptant periodic procein membane 1 NM_003010 H5.234011 2.616	82	-	ensembl genscan prediction	AL163932	-	chromosome 14	2.846	85.7%	EST
8 XVD3 Virtual duratin scenario regulator 3 NT007071 B. SU150 [9] 11.1.1.1.12 2.70 8.75 realabilian 87 RAB1/A rable in scenario regulator 3 NT00744 B.5371 1521.3.q22.3 2.70 8.75 realabilian 87 RAB1/A rable in rember riso oncogene family NL005805 H.537141 15q21.3.q22.3 2.70 8.75 metabolian 88 PMD1/A 255 proteasome associated pail bounding NL005805 H.537140 2424 2.80 8.74 metabolian 9 RJ erinoblations of pon realing famil 12 NL001715 H.548060 921.3 2.80 8.74 metabolian 9 LOC52204 thypothesial LOC54202 NL001001 H.53060 171.12 2.80 8.75 metabolian 9 LOC52204 thypothesial LOC54202 NL001001 H.53060 921.3 2.404 8.75 metabolian 9 LOC52204 pond Hap405 bounding, subfamily C, member 7 NL01301 H.530627 1225.4 8.75	83	PP	pyrophosphatase (inorganic)	NM_021129	Hs.437403	10q11.1-q24	2.837	85.7%	metabolism
86 CS/977 using specific perplica 47 NN_017944 Hs.S7521 Lip153 2.70 8.79 metabolian (metabolian specific perplica 47) 87 RABITA rabla, member as oncogene family NM_00360 Hs.371541 15(21-3)42.32 2.70 8.79 metabolian (metabolian specific perplica 47) 88 PMD14 20x protesome-associated path benolog NM_00321 Hs.567410 24/22 2.714 8.73* metabolian (metabolian family compatibility) 90 RTT reintoblastoma I NM_00321 Hs.450760 22121 2.201 8.73* estT 91 ATP281 hsystome patics, cirt randolian metabolian (metabolian (metabolian protein histome NM_00321 Hs.50164 16912.3 2.601 8.73* estT 92 DAJCGP 20166 protein XXL290070 Hs.51644 16912.3 2.608 8.74* estToblian 93 readbolian protein initiane XXL290070 Hs.51642 16912.3 2.648 8.74* estToblian initian (matabolian metabolian metabolian initiane) 94 RAAC72 KALA0	84	CYCS	cytochrome c	NM_018947	Hs.437060	7p15.3	2.792	85.7%	mitochondrion
87 84.01/A no.11 a, number rac oncogene family NA.01466 15.321.34 15.921.342.32 2.50 8.50 15.000000000000000000000000000000000000	85	FXYD3	fxyd domain-containing ion transport regulator 3	BT006712	Hs.301350	19q13.11-q13.12	2.769	85.7%	metabolism
Image: Instance of the	86	USP47	ubiquitin specific peptidase 47	NM_017944	Hs.567521	11p15.3	2.760	85.7%	metabolism
88 SMD14 256 protasome-sasceind pall henologi NNL 00592 H: 54714 34212 27.4 87.5 ortholian 9 RD1 reinoolasome 13 pen reading frame 12 NNL 01951 H: 54874 134123 2.60 87.5 cell polification 9 RD72B1 appase.cu ⁺ ransporting.pitama membrate NML 00183 H: 50050 F1213 2.605 87.5 cell polification 9 RD7 PML/C7 Dod (H:pd0) fonnolog, subfamily C, member NML 00183 H: 50050 F1712 2.66 87.5 estate 9 RD7 RE66 pensation protein kinase NML 01497 H: 19771 49.33.24.11 2.61 87.5 metabolism 9 - ensation protein kinase NML 01497 H: 19771 49.33.24.11 2.62 88.75 metabolism 9 - ensation protein kinase NML 01497 H: 198771 49.33.24.11 2.52 87.5 metabolism 9 - ensation protein kinase NML 01493 H: 18.9877 H: 18.9877 </td <td>87</td> <td>RAB11A</td> <td>rab11a, member ras oncogene family</td> <td>NM_004663</td> <td>Hs.321541</td> <td>15q21.3-q22.31</td> <td>2.750</td> <td>85.7%</td> <td>signal</td>	87	RAB11A	rab11a, member ras oncogene family	NM_004663	Hs.321541	15q21.3-q22.31	2.750	85.7%	signal
80 71.0m/2 1.5.26%74 1.5.12 2.9.1 8.7.3 edip selfication 90 RID reinoblatoma 1 NL_01032 Hs.40523 1.5.12.3 2.69 8.7.3 edip selfication 91 AT2281 apue. cs ^{**} transporting. plasm amerbera 1 NL_010121 Hs.50027 1.24.12 2.69 8.7.3 enabolian 92 DAX Dax (HzPo) homolog. sublanity C.member 7 NL_01037 Hs.51060 1.91.2 2.69 8.7.8 metabolian 97 FIB.6 WAA0220-like protein XL_20070 Hs.5167 1.91.2 2.69 8.7.8 metabolian 97 FIB.6 weakly similar to fibulin-1 isoform d presume AK02744 Hs.58877 1.92.32 2.52 8.578 metabolian 90 S.7 resonal protein s7 NL_00130 Hs.54037 2.91.1-12 2.65 8.7.9 FET 91 S.7 resonal protein s0rein fistone AK02744 Hs.25877 1.92.32 8.7.9 metabolian 92 S.7 <td< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>transduction</td></td<>									transduction
8) Clorigit serials Nu.0193 His.28742 Japl.23 2.00 87.9 edip 91 ATP2B1 atpase.cl* transporting.japana membrane1 Nu.00168 His.0825 Japl.21 2.60 87.9 Barl 91 ACC35420 Dead(1450) (homelog. subfinit), Cmmethr Nu.010168 His.08206 Pol.13 2.60 85.7% Barl 91 DAU KIAAD220-like protein Nu.01977 His.31660 Ipl.12 2.67 87.9% Indente 91 FIB.6 MIAD20-like protein Nu.01977 His.31670 1pl.32.41 2.68 87.9% Indente 92 FIB.6 washy similar to fibrin-1 lisform d precursor AK07240 His.83877 1pl.32.41 2.68 87.9% Indente 93 R7 manal protein s7 Nu.01370 His.43823 2.121-122 2.58 87.9% Indente 94 R74 savata macha protein s7 Nu.01370 His.43823 1pl.32.1 2.51 His.31660 Ipl.32.1 8.78 His.31660 95 ratio savata macha protein sortin 35 Nu.01370 His.43823 1pl.32.1 2.51 His.3166 His.31671 1pl.32.1 His.31671 His.31671 <td< td=""><td>88</td><td>PSMD14</td><td>26s proteasome-associated pad1 homolog</td><td>NM_005805</td><td>Hs.567410</td><td>2q24.2</td><td>2.724</td><td>85.7%</td><td>metabolism</td></td<>	88	PSMD14	26s proteasome-associated pad1 homolog	NM_005805	Hs.567410	2q24.2	2.724	85.7%	metabolism
91 <i>JP2211 Jpspectact Jpsppectact Jpspectact</i> <	89	C13orf12	chromosome 13 open reading frame 12	NM_015932	Hs.268742	13q12.3	2.698	85.7%	others
91 47/211 apps. a" transporting plasma membrane in	90	RB1	retinoblastoma 1	NM_000321	Hs.408528	13q14.2	2.691	85.7%	cell proliferation
92 DC35420 pond (idsp40) homolog, subfamily C, member 7 NC11715 HL 548906 9, 21, 3 2, 67 8, 77 membalism 93 DMA/C7 Dmal (idsp40) homolog, subfamily C, member 7 NL 00303 His 50106 16p12.3 2, 687 8, 57% entroll 94 RLA0220 KLA0220-like protein NL 020600 - chronosome 1 2, 64 8, 75% entroll 8, 75% 167 7, 77, 77, 77 7, 77, 77 8, 75% entroll entroll 8, 75% entroll 8, 75% entroll entroll 1, 65, 4687 1, 22, 12, 122 2, 55% entroll entroll 100 FLZ entroll resclated nuclear protein 7 NL, 00110 His 546237 7, 22, 12, 122 2, 55% entroll 1, 62, 12, 122 2, 55% entroll 1, 62, 12, 122 55% N metabalism 101 FLZ Sprotein fortin 12, 56, 12 ALD31186 - 2, 72, 12, 122 55, 7% metabalism 102 Sprotein protein 12, 56, 12 NM, 00120 His, 4022<	91	ATP2B1	atpase, ca ⁺⁺ transporting, plasma membrane 1			-		85.7%	-
93 DNALC7 Dmail (Hsp40) homolog, subfamily C, member 7 NM_00315 Hs 50156 I (11 2) 2.687 8.57% metabolism 94 KIAA0220 KIAA0220-like protein AC02200 - chromosome 1 2.658 8.57% others 95 - mesmbl genesan prediction AC02200 - chromosome 1 2.661 8.57% metabolism 97 FIRL 6 weakly similar to fluin-1 isoform d precursor AK02744 His5877 1923-3-411 2.618 8.57% metabolism 98 R/97 ribosomal protein 37 NL001101 His 54807 7.21 2.568 8.57% metabolism 99 - ensembl genesan prediction AL031186 - 2.212.11/2.2 2.55% Rediobing 101 FL/22662 NL001301 His 48400 7.213.1 2.518 8.57% metabolism 102 RA sorcin ras-related maclear protein sorting 3.5 (yeas) NL018031 His 1082 1213.1 2.518 8.57% metabolism						-			
95.ensembl genom predictionAC022900.chromoxom2.6548.75787596NEK6pundriv serine-incomis protein kinaseNL014397HL017093.3241.12.168.75inclubilism98RPS7ribosomal protein s7NL01101HL528-3712.253.12.5888.707inclubilism99.ensembl gensan predictionNL031186.2.2412.1-1222.5658.77inclubilism100RFIsorvinNL031186.2.2412.1-1222.5658.77inclubilism101FLZ 202hypothetical protein fl22602NL004289HL131931.218.12.5148.78inclubilism101FLZ 202hypothetical protein fl22602NL004289HL131931.218.12.5148.78inclubilism102RAWracelated malcar proteinNL01208HL5452816q122.5188.77inclubilism103JCCode protein sorting 35 (yeast)NL01208HL5452816q122.198.78inclubilism104VPS35vacular protein sorting 15NL016106HL501881191.52.4888.77inclubilism105.ensemb gensaem prediction rA9NL016106HL501881191.22.4798.77inclubilism105.SCFD1sectar function infigurant rA9NL016106HL501881191.22.4798.78inclubilism106PSCF1sectar function infigurant rA9NL0161			•						
95.ensembl general predictionAC02 6900.chromonoum2.6548.75787596NEX60nutrive serice-inconing protein kinaseNM.014397HS.98771923-3-3112.168.75816bolism98RPS7ribosomal protein s7NM.01010HS.98771923-3-3112.168.758751000000000000000000000000000000000000	94	KIAA0220	KIAA0220-like protein	XM 290670	Hs.531664	16p12.3	2.659	85.7%	others
96 NEK6 patate			<u>^</u>			<u>^</u>			
97 <i>FBL</i> .6 weakly similar to fibulin-1 isoform d precursor AK027344 Hs.5877 Jq253.q31.1 2.412 8.57% intrabalation and processing 98 <i>RPS7</i> nosconal protein s7 NM_001011 Hs.5887 Jq25 2.58 8.7% intrabalation and processing 99 - ensembl genscan prediction AL011186 - 22q12.11.2 2.565 8.7% ERT 101 <i>FL22660</i> hypothetical protein fij2662 NM_00128 Hs.19433 12p13.1 2.541 8.57% renabolism and processing 103 <i>DC6</i> do protein MM_00128 Hs.49255 8q23.1 2.518 85.7% renabolism and processing 104 VPS35 vacuolar protein sorting 35 (yeast) NM_01206 Hs.49275 5q35.2 2.48 85.7% metabolism 105 <i>LAMAI 542</i> CTD-binding Rs.like protein rA9 NM_01606 Hs.492175 5q35.2 2.48 85.7% metabolism 106 <i>KLAMI 542</i> CTD-binding Rs.like protein rA9 NM_016106 Hs.491781		NEK6			Hs.197071				
98 <i>RPST</i> ribosomal protein s7 NM_00101 Hs.54287 2 p25 2.88 8.7.% ranslation and processing and protein spectrom s			* *						
99 . ensembl genscan prediction AL031186 . 22q12.1-12.2 255 85.7% EST 101 <i>FLJ22662</i> hypothetical protein fj22662 NM_003130 Hs.480040 7q21.1 2.521 85.7% EST 102 <i>RAN</i> ras-related nuclear protein NM_00525 Hs.10842 12q13.1 2.541 85.7% EST 103 <i>DC6</i> def protein arrowing 15 (yeast) NM_00525 Hs.10842 12q12.1 2.519 85.7% real processing 104 <i>VPS35</i> vacuolar protein sorting 25 (yeast) NM_018206 Hs.45428 16q12 2.19 85.7% metabolism 105 - ensembl genscan prediction AC080053 - chromosome 11 2.512 85.7% EST 106 <i>KIAAL542</i> CTD-binding SR-like protein rA9 NM_016106 Hs.369168 14q12 2.49 85.7% metabolism 107 <i>HSPC111</i> hypothetical protein fA9 NM_014264 Hs.1302 1721.2 2.46 85.7% transcripti			•						
100 SRI sorcin NM_003130 Hs.489040 7q21.1 2.521 85.7% metabolism 101 F/122662 hypothetical protein fij22662 NM_02429 Hs.131933 12p1.1 2.541 85.7% cell protiferation 103 DC6 dc6 protein NM_002189 Hs.492555 8q23.1 2.531 85.7% cell protiferation 104 VPS35 vacuolar protein sorting 35 (yeast) NM_018206 Hs.454528 I6q12 2.519 85.7% metabolism 105 - ensembl genscan prediction AC080053 - chromosome 11 2.512 85.7% metabolism 107 HSPC111 hypothetical protein HSPC111 NM_016301 Hs.529475 5q35.2 2.48 85.7% metabolism 108 SCFD1 sec1 family domain containing 1 NM_004278 Hs.499790 17p12-p11.2 2.476 85.7% metabolism 110 LSM3 LSM3 homolog. U6 small nuclear RNA associated NM_004278 Hs.181052 12p21.1 2.441 85.7%	20	ni s,		1111_001011	115.5 10207	2923	2.500	05.770	
101 <i>FL/22662</i> hypothetical protein flj22662 NM_024829 Hs.13133 1.2p1.3.1 2.541 8.5.7% eBT 102 <i>RAN</i> ras-related nuclear protein NM_006225 Hs.10842 1.2q24.3 2.541 8.5.7% etal proliferation 103 <i>DC6</i> de forotein NM_002109 Hs.492555 8.231 2.510 8.5.7% transcription 104 <i>VPS15</i> vacuolar protein sorting 35 (yeast) NM_018206 Hs.492552 6.321 2.512 8.5.7% transcription 105 - esembli genscan prediction ACM08053 - chromosone 11 2.128 8.5.7% intracellular 107 <i>HSPC111</i> hypothetical protein HSPC111 NM_0016391 Hs.529475 5q32.2 2.488 8.5.7% intracellular 108 <i>SCFD1</i> sect family domain containing 1 NM_016106 Hs.369168 14q12 2.479 8.5.7% intracellular 109 <i>PIGL</i> phosphatidylinositol glycan, class 1 NM_004278 Hs.490793 17p12-p11.2 2.46 8.5.7% intracellular 110 <i>LSM3</i>	99	-	ensembl genscan prediction	AL031186	-	22q12.1-12.2	2.565	85.7%	EST
102 RAN ras-related nuclear protein NM_006325 Hs.10842 12q24.3 25.34 85.7% cell proliferation 103 DC6 de6 protein NM_02018 Hs.492555 Rel22.1 2.51 85.7% metabolism 104 VFS35 vacuolar protein sorting 35 (ycast) NM_018206 Hs.454528 I6q12 2.51 85.7% metabolism 105 - ensembl genscan prediction AC080033 - chromosome 11 2.51 85.7% metabolism 106 KIAA/2 CTD-binding SR-like protein A9 NM_001001 Hs.529475 5q35.2 2488 85.7% metabolism 107 HSPC111 hypothetical protein HSPC111 NM_016106 Hs.499793 17p12-p11.2 2.45 85.7% metabolism 108 SCFD1 secl family domain containing 1 NM_004278 Hs.499793 17p12-p11.2 2.45 85.7% metabolism 110 LSM3 bmobg, U small nuclear RNA associated NM_014708 Hs.499793 17p12-p11.2 2.45 85.7% mascription 1110 LMG7 motabolism protein MSN	100	SRI	sorcin	NM_003130	Hs.489040	7q21.1	2.552	85.7%	metabolism
103 DC6 de6 protein NM_02019 Hs.492555 8q23.1 2.51 8.7.% transcription and processing 104 VFS35 vacuolar protein sorting 35 (yeast) NM_018206 Hs.454528 16q12 2.519 85.7% realbolism 105 ensembl genscan prediction AC080053 chromosome11 2.512 85.7% realbolism 106 KIAAI542 CTD-binding SR-like protein rA9 NM_020001 Hs.329838 11p15.5 2.489 85.7% realbolism 107 HSPC111 hypothetical protein HSPC111 NM_010601 Hs.369168 14q12 2.479 85.7% metabolism 108 SCFD1 sec1 family domain containing 1 NM_010460 Hs.499793 17p12-p11.2 2.451 85.7% metabolism 109 PIGL phosphatidylinositol glycan, class 1 NM_01460 Hs.11632 3p25.1 2,454 85.7% metabolism 1110 LCG389651 similar to hypothetical protein (L1H 3 region) XM_372039 Hs.567978 8p11.1 2.434 85.7%	101	FLJ22662	hypothetical protein flj22662	NM_024829	Hs.131933	12p13.1	2.541	85.7%	EST
104 VPS35 vacuolar protein sortig 35 (yeast) NM_018206 Hs.454528 I fol 2 2.519 85.7% metabolism 105 - ensembl genscan prediction AC080053 - chromosome 11 2512 85.7% EST 106 KIA.41542 CTD-binding SR-like protein rA9 NM_002001 Hs.252838 11p15.5 2.489 85.7% metabolism 107 HSPC111 hypothetical protein HSPC111 NM_016106 Hs.359168 14q12 2.479 85.7% metabolism 108 SCFD1 secl family domain containing 1 NM_014463 Hs.111632 3p25.1 2.451 85.7% metabolism 110 LSM3 LSM3 bomolog, U6 small nuclear RNA associated NM_014463 Hs.111632 3p25.1 2.451 85.7% transcription and processing 111 WDR61 WD repeat domain 61 AF30953 Hs.513055 15q25.1 2.451 85.7% transcription and processing 112 LOC389651 similar to hypothetical protein (L1H 3 region) XM_372039 Hs.437656 <td>102</td> <td>RAN</td> <td>ras-related nuclear protein</td> <td>NM_006325</td> <td>Hs.10842</td> <td>12q24.3</td> <td>2.534</td> <td>85.7%</td> <td>cell proliferation</td>	102	RAN	ras-related nuclear protein	NM_006325	Hs.10842	12q24.3	2.534	85.7%	cell proliferation
104 VFS35 vacuolar protein sorting 35 (yeast) NM_018206 Hs.454528 16q12 2.519 85.7% metabolism 105 — ensembl genscan prediction AC080053 — hromosome 11 2.512 85.7% FST 106 KIAAL742 CTD-binding SR-like protein rA9 NM_020901 Hs.325838 11p15.5 2.488 85.7% intracellular organelle 107 HSPC11 hypothetical protein HSPC111 NM_016109 Hs.499793 17p12-p11.2 2.465 85.7% metabolism 108 SCFD1 secl family domain containing 1 NM_004278 Hs.499793 17p12-p11.2 2.465 85.7% metabolism 109 PIGL psophatidylinositol glycan, class 1 NM_004278 Hs.11632 3p25.1 2.451 85.7% transcription and processing 110 LSM3 ismilar to hypothetical protein (1.1H 3 region) XM_37203 Hs.513055 15q25.1 2.451 85.7% EST 113 LEPRE1 growh suppressor 1 NM_0202236 Hs.437656 Ip	103	DC6	dc6 protein	NM_020189	Hs.492555	8q23.1	2.531	85.7%	<u>^</u>
105 ensembl genscan prediction AC080053 ehromosome 11 2.512 85.7% EST 106 KIAAI542 CTD-binding SR-like protein rA9 NM_020901 Hs.325838 11p15.5 2.489 85.7% metabolism 107 HSPC111 hypothetical protein HSPC111 NM_016391 Hs.529475 5q35.2 2.488 85.7% metabolism 108 SCFD1 secl family domain containing 1 NM_016106 Hs.699793 17p12-p11.2 2.465 85.7% metabolism 109 PIGL phosphatidylinositol glycan, class I NM_004278 Hs.49793 17p12-p11.2 2.454 85.7% metabolism 110 LSM3 LSM3 bomolog, U6 small nuclear RNA associated NM_014463 Hs.111632 3p25.1 2.451 85.7% framscription and processing 111 WDR61 WD repeat domain 61 AF309553 Hs.51755 5q21.1 2.454 85.7% framscription and processing 112 LOC389651 similar to hypothetical protein (L1H 3 region) XM_372039 Hs.518718 17q2.1	104	LUDG25	1	NR 010206	11 45 4520	16.10	2 5 1 0	05 70	· ·
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118SEC61Gsec61 gammaNM_014302Hs.4882827p11.22.37885.7%intracellular organelle119SSBP1single-stranded dna-binding proteinNM_003143Hs.4903947q342.36985.7%mitochondrion120C14orf112chromosome 14 open reading frame 112NM_016468Hs.13710814q24.22.36785.7%others121ATP5Eatp synthase, h+ transporting, mitochondrial f1 complex, epsilon suburitNM_000166Hs.19750020q13.322.36285.7%mitochondrion123ETFAelectron transfer flavoprotein, alpha polypeptideNM_000126Hs.3992515q23-q252.34585.7%metabolism123TBX4t-box 4NM_00126Hs.14390717q21-q222.32885.7%itanscription124H3F3AH3 histone, family 3ANM_002107Hs.5336241q412.32885.7%intracellular organelle	117	ARPC2	actin related protein 2/3 complex, subunit 2	NM_005731	Hs.529303	2q36.1	2.380	85.7%	· ·
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122 ETFA electron transfer flavoprotein, alpha polypeptide NM_000126 Hs.39925 15q23-q25 2.345 85.7% metabolism 123 TBX4 t-box 4 NM_018488 Hs.143907 17q21-q22 2.328 85.7% transcription and processing 124 H3F3A H3 histone, family 3A NM_002107 Hs.533624 1q41 2.328 85.7% intracellular organelle	120	C14orf112	chromosome 14 open reading frame 112	NM_016468	Hs.137108	14q24.2	2.367	85.7%	others
122 ETFA electron transfer flavoprotein, alpha polypeptide NM_000126 Hs.39925 15q23-q25 2.345 85.7% metabolism 123 TBX4 t-box 4 NM_018488 Hs.143907 17q21-q22 2.328 85.7% transcription and processing 124 H3F3A H3 histone, family 3A NM_002107 Hs.533624 1q41 2.328 85.7% intracellular organelle	121	ATP5E		NM_006886	Hs.177530	20q13.32	2.362	85.7%	mitochondrion
123 TBX4 t-box 4 NM_018488 Hs.143907 17q21-q22 2.328 85.7% transcription and processing 124 H3F3A H3 histone, family 3A NM_002107 Hs.533624 1q41 2.328 85.7% intracellular organelle	122	ETEA	* *	NM 000126	Hs 30025	15023-025	2 345	85 70%	metabolism
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124 H3F3A H3 histone, family 3A NM_002107 Hs.533624 1q41 2.328 85.7% intracellular organelle	123	104	100A 7	14141_010400	115.143707	1/421-422	2.320	05.170	-
organelle	124	ЦЗЕЗА	U3 histone family 3A	NM 002107	U. 522624	1:41	2 2 2 9	85 701	· •
-	124	пэґ зА	no instone, family SA	INIM_002107	HS.333624	1941	2.328	83.1%	
	125	PPIA	peptidylprolyl isomerase a (cyclophilin a)	NM_021130	Hs.356331	7p13-p11.2	2.319	85.7%	-

No.	Symbol	Gene name	Accession	UniGene	Location	Median	% Up	Function
126	ZNF84	zinc finger protein 84 (HPF2)	NM_003428	Hs.445019	12q24.33	2.293	85.7%	transcription and processing
127	LOC400451	hypothetical gene supported by AK075564; BC060873	NM_207446	Hs.27373	15q26.1	2.279	85.7%	EST
128	MAPK8IP2	mitogen-activated protein kinase 8 interacting protein 2	NM_012324	Hs.356523	22q13.33	2.261	85.7%	signal transduction
129	MDH2	malate dehydrogenase 2, nad (mitochondrial)	NM_005918	Hs.520967	7p12.3-q11.2	2.202	85.7%	mitochondrion
130	RNF39	ring finger protein 39	AF238317	Hs.121178	6p21.3	2.159	85.7%	metabolism
131	UCRC	ubiquinol-cytochrome c reductase complex	NM_013387	Hs.284292	22cen-q12.3	2.153	85.7%	mitochondrion
132	CSE1L	cse1 chromosome segregation 1-like (yeast)	NM_001316	Hs.90073	20q13	2.127	85.7%	mitochondrion
133	C6orf49	chromosome 6 open reading frame 49	NM_013397	Hs.525899	6p21.31	2.064	85.7%	metabolism
134	PSMA7	proteasome (prosome, macropain) subunit, alpha type, 7	NM_002792	Hs.233952	20q13.33	2.063	85.7%	metabolism
135	C8orf17	chromosome 8 open reading frame 17	AF220264	Hs.283098	8q24.3	2.023	85.7%	cell proliferation
136	SERP1	stress-associated endoplasmic reticulum protein 1	NM_014445	Hs.518326	3q25.1	2.009	85.7%	intracellular organelle

Table II. Continued.

Statistical analysis and annotation of gene function. Relationships between the 2 groups and the numerical values obtained by real-time RT-PCR were analyzed by the Mann-Whitney U test. Relationships among the 3 groups and the numerical values were analyzed by the Bonferroni-adjusted Mann-Whitney U test. The analysis software was Expert StatView (version 4, SAS Institute Inc., Cary, NC, USA); for comparison tests among the 3 groups, the non-adjusted statistical level of significance (p<0.05) corresponds to a Bonferroni-adjusted statistical significance of p<0.0167.

The molecular function of the up- and down-regulated genes was classified into 13 groups as referenced in GENEONTOLOGY (http://www.geneontology.org/) and GeneCards (http://www.genecards.org/index.shtml), i.e. metabolism, transcription and processing, translation and processing, signal transduction, cell proliferation, cell-cycle regulation, cell differentiation, cell adhesion/surface-linked, cytoskeleton/cell membrane-linked, intracellular organelle, mitochondrion, other, and expressed sequence tags (ESTs) (18).

Results

Identification of genes expressed differently in BC and normal bladder. By microarray analysis of 14 BCs we identified 136 genes that were generally up-regulated more than 1.5-fold in BC compared to normal bladder (Table II). Among these, 21 were up-regulated in all 14 BCs (100%), 44 in 13 (92.9%), and the other 71 were up-regulated in 12 BCs (85.7%). On the contrary, 69 genes were down-regulated less than -1.5-fold (Table III). Among these, 25 were down-regulated in all 14 BCs (100%), 22 in 13 (92.9%), and the other 22 in 12 BCs (85.7%).

Molecular function of up- and down-regulated genes in BC. The functional annotation of the 136 up- and 69 downregulated genes is presented in Fig. 1. Functionally, the upregulated genes were involved in metabolism (36%), trans-

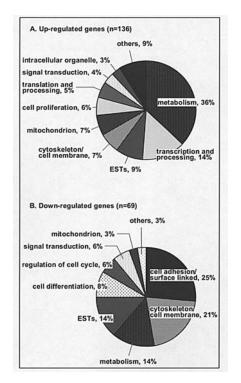


Figure 1. Distribution of the expression of functionally categorized genes in human BC. The functional features of the 136 up- (A) and 69 down-regulated genes (B) were classified into 13 categories.

cription and processing (14%), ESTs (9%), cytoskeleton/cell membrane (7%), mitochondrion (7%), cell proliferation (6%), translation and processing (5%), signal transduction (4%), intracellular organelle (3%), and other functions (9%) (Fig. 1A). The down-regulated genes were involved in cell adhesion/surface linked (25%), cytoskeleton/cell membrane (21%), metabolism (14%), ESTs (14%), cell differentiation (8%), cell-cycle regulation (6%), signal transduction (6%), mito-chondrion (3%), and other functions (3%) (Fig. 1B).

No.	Symbol	Gene name	Accession	UniGene	Location	Median	% Up Dowr	Function
1	TAGLN	transgelin	NM_003186	Hs.503998	11q23.2	0.095	100.0%	cytoskeleton/
2	TPM2	tropomyosin 2 (beta)	NM_003289	Hs.300772	9p13.2-p13.1	0.113	100.0%	cell membrane cytoskeleton/ cell membrane
3	MGP	matrix Gla protein	NM_000900	Hs.365706	12p13.1-p12.3	0.121	100.0%	cell adhesion/ surface linked
4	GSN	gelsolin	NM_198252	Hs.522373	9q33	0.147	100.0%	cytoskeleton/ cell membrane
5	CNN1	calponin 1, basic, smooth muscle	NM_001299	Hs.465929	19p13.2-p13.1	0.161	100.0%	cytoskeleton/ cell membrane
6	MYLK	myosin light chain kinase, isoform 6	NM_005965	Hs.477375	3q21	0.164	100.0%	metabolism
7	DF	adipsin/complement factor d precursor	NM_001928	Hs.155597	19p13.3	0.168	100.0%	metabolism
8	TPM1	tropomyosin 1 (alpha)	AL050179	Hs.133892	15q22.1	0.170	100.0%	cytoskeleton/
					*			cell membrane
9	FN1	fibronectin 1	NM_002026	Hs.203717	2q34	0.171	100.0%	cell adhesion/ surface linked
10	DES	desmin	NM_001927	Hs.471419	2q35	0.176	100.0%	cytoskeleton/ cell membrane
11	ILK	integrin-linked kinase	NM_004517	Hs.5158	11p15.5-p15.4	0.192	100.0%	signal transduction
12	DCN	decorin	NM_001920	Hs.156316	12q21.33	0.206	100.0%	cell adhesion/ surface linked
13	CKB	creatine kinase, brain	NM_001823	Hs.173724	14q32	0.206	100.0%	metabolism
14	MYL9	myosin regulatory light chain 2, smooth muscle isoform	NM_006097	Hs.504687	20q11.23	0.206	100.0%	cytoskeleton/ cell membrane
15	FBLN5	fibulin 5	NM_006329	Hs.332708	14q32.1	0.236	100.0%	cell adhesion/ surface linked
16	DSTN	destrin (actin depolymerizing factor)	NM_006870	Hs.304192	20p12.1	0.244	100.0%	cell adhesion/ surface linked
17	SFRP2	secreted frizzled-related protein 2	BC008666	Hs.481022	4q31.3	0.250	100.0%	regulation of cell cycle
18	FXYD6	FXYD domain containing ion transport regulator 6	NM_022003	Hs.504031	11q23.3	0.262		cytoskeleton/ cell membrane
19	CRYAB	crystallin, alpha b	NM_001885	Hs.408767	11q22.3-q23.1	0.274	100.0%	others
20	IGFBP7	insulin-like growth factor binding protein 7	NM_001553	Hs.479808	4q12	0.276	100.0%	regulation of cell cycle
21	COL6A2	type vi collagen alpha 2 chain precursor	AY029208	Hs.420269	21q22.3	0.334	100.0%	cell adhesion/ surface linked
22	LGALS1	lectin, galactoside-binding, soluble, 1 (galectin 1)	BC001693	Hs.445351	22q13.1	0.335	100.0%	cell differentiation
23	BCR	bcr-abl mrna 5' fragment clone 3c; unknown protein 77 aa	X14675	Hs.517461	22q11	0.335	100.0%	regulation of cell cycle
24	MGLL	monoglyceride lipase	NM_007283	Hs.277035	3q21.3	0.356	100.0%	metabolism
25	HSPC072	HSPC072 protein	NM_014162	Hs.439352	20p11.23	0.407	100.0%	EST
26	CAV1	caveolin 1	NM_001753	Hs.74034	7q31.1	0.180	92.9%	cytoskeleton/ cell membrane
27	CLEC3B	C-type lectin domain family 3, member B	NM_003278	Hs.476092	3p22-p21.3	0.206	92.9%	cytoskeleton/ cell membrane
28	COL1A2	alpha 2 type i collagen preproprotein	NM_000089	Hs.489142	7q22.1	0.211	92.9%	cell adhesion/ surface linked
29	CLU	clusterin	NM_001831	Hs.436657	8p21-p12	0.230	92.9%	EST
30	PLA2G2A	phospholipase a2, group iia (platelets, synovial fluid)	NM_000300	Hs.466804	1p35	0.230	92.9 <i>%</i> 92.9%	metabolism
30 31	COL3A1	alpha 1 type iii collagen preproprotein	NM_000090	Hs.443625	2q31	0.247	92.9% 92.9%	cell adhesion/ surface linked
32	COL1A1	collagen, type I, alpha 1	NM_000088	Hs.172928	17q21.3-q22.1	0.274	92.9%	cell adhesion/ surface linked
33	IGFBP6	insulin-like growth factor binding protein 6	NM_002178	Hs.274313	12q13	0.295	92.9%	regulation of cell cycle

Table III. Continued.

No.	Symbol	Gene name	Accession	UniGene	Location	Median	% Up Dowr	n Function
34	HBA1	hemoglobin, alpha 1	AF281258	Hs.449630	16p13.3	0.296	92.9%	mitochondrion
35	FHL1	four and a half lim domains 1	NM_001449	Hs.435369	Xq26	0.308	92.9%	cytoskeleton/
	BB (1) (1)			XX 0.50(2)	4.95	0.015	00.007	cell membran
36	PDLIM3	alpha-actinin-2-associated lim protein	NM_014476	Hs.85862	4q35	0.317	92.9%	cytoskeleton/
37	AEBP1	adipocyte enhancer binding protein 1 precursor	NM_001129	Hs.439463	7p13	0.350	92.9%	cell membran
01		adipolyte children official protein 1 precursor	1001129	115.159 105	, p15	0.550	12.17	surface linked
38	C1R	complement component 1, r subcomponent	NM_001733	Hs.524224	12p13	0.376	92.9%	metabolism
39	PCP4	purkinje cell protein 4	NM_006198	Hs.80296	21q22.2	0.222	85.7%	metabolism
40	CTSK	cathepsin k (pycnodysostosis)	NM_000396	Hs.523594	1q21	0.386	92.9%	cell adhesion/
								surface linked
41	ACTG1	actin, gamma 1	BC004223	Hs.514581	17q25	0.416	92.9%	cytoskeleton/
10	DTCDC		NIM 000054	11. 446420	0-24.2 -24.2	0.410	02.001	cell membran
42	PTGDS	prostaglandin d2 synthase (21kd, brain)	NM_000954	Hs.446429 Hs.25338	9q34.2-q34.3	0.418	92.9%	metabolism
43 44	PRSS23 CKIP-1	putative secreted protein zsig13 ck2 interacting protein 1; hq0024c protein; loc51177	AF193611 NM_016274	Hs.438824	11q14.1 1q21.2	0.421 0.435	92.9% 92.9%	EST EST
45	TIMP1	tissue inhibitor of metalloproteinase 1 precursor	NM_003254	Hs.522632	Xp11.3-p11.23	0.453	92.9% 92.9%	cell adhesion/
			100201	1101022002	iipiilo piilo	01100	/ 20/10	surface linked
46	KLF3	Kruppel-like factor 3 (basic)	NM_016531	Hs.298658	4p14	0.520	92.9%	signal
					[*]			transduction
47	-	ensembl genscan prediction	AC007346	-	chromosome 16	0.466	92.9%	EST
48	MYH11	myosin, heavy polypeptide 11, smooth muscle	NM_022844	Hs.460109	16p13.13-p13.12	0.266	85.7%	celladhesion/
								surface linked
49	ITM2A	integral membrane protein 2a	NM_004867	Hs.17109	Xq13.3-Xq21.2	0.318	85.7%	cell
50					14.00	0.000	05.50	differentiation
50	-	alpha heavy chain	X17116	-	14q32	0.323	85.7%	EST
51	RGS2	regulator of g-protein signalling 2, 24kd	NM_002923	Hs.78944	1q31	0.355	85.7%	signal transduction
52	COL6A2	collagen, type VI, alpha 2	BC002484	Hs.420269	21q22.3	0.370	85.7%	cell adhesion/
52	COLONE	conagon, type vi, apia 2	BC002101	113.120209	21922.0	0.570	05.170	surface linked
53	VWF	von willebrand factor	NM_000552	Hs.440848	12p13.3	0.376	85.7%	cell adhesion/
								surface linked
54	FBLN1	fibulin 1 isoform d	NM_006486	Hs.24601	22q13.31	0.376	85.7%	cell adhesion/
								surface linked
55	SERPINF1	serine (or cysteine) proteinase inhibitor,	NM_002615	Hs.532768	17p13.1	0.409	85.7%	cell
		clade f member 1						differentiation
56 57	-	nc_001807 mitochondrion complete genome	NC_001807	-	mitochondrion	0.414	85.7%	mitochondrio
57	EFEMP1	egf-containing fibulin-like extracellular matrix protein 1, isoform b	NM_018894	Hs.76224	2p16	0.425	85.7%	cell adhesion/ surface linked
58	COX7A1	cytochrome c oxidase subunit viia polypeptide 1	NM_001864	Hs.421621	19q13.1	0.431	85.7%	metabolism
50	COMM	(muscle)	1111_001004	113.421021	1)415.1	0.451	05.170	metabolism
59	CSRP1	cysteine and glycine-rich protein 1	NM_004078	Hs.108080	1q32	0.433	85.7%	cell
					•			differentiation
60	HBD	hemoglobin, delta	NM_000519	Hs.36977	11p15.5	0.451	85.7%	metabolism
61	RPS6KA5	rsk-like protein kinase rlpk	AF080000	Hs.510225	14q31-q32.1	0.465	85.7%	signal
								transduction
62	SPRR3	small proline-rich protein 3	AJ243667	Hs.139322	1q21-q22	0.483	85.7%	cell
								differentiation
63	TBX1	t-box 1 transcription factor c	AF373867	Hs.173984	22q11.21	0.484	85.7%	others
64 65	- SPARC	ensembl genscan prediction secreted protein, acidic, cysteine-rich (osteonectin)	AF277315	- He 111770	Xq28	0.484	85.7% 85.7%	EST
65	SI AKC	scereieu protein, acture, cystellie-fich (osteollectifi)	NM_003118	Hs.111779	5q31.3-q32	0.484	05.170	cell adhesion/ surface linked
66	_	ensembl genscan prediction	AC007601	-	chromosome 16	0.509	85.7%	EST
67	SVIL	supervillin, isoform 1	NM_003174	Hs.499209	0.526	85.7%		
		•						differentiation
68	KIAA0582	kiaa0582 protein	NM_015147	Hs.146007	2p14	0.536	85.7%	EST
69	-	hypothetical protein FLJ20186	NM_207514	Hs.62771	16q24.3	0.565	85.7%	EST

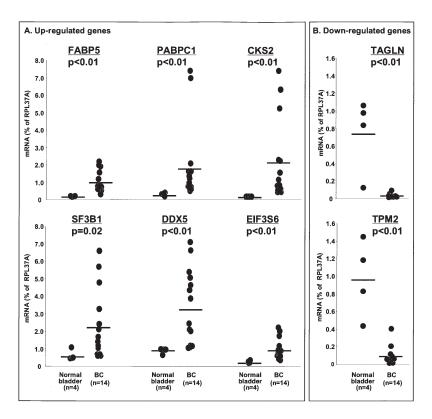


Figure 2. Comparison of the real-time PCR results for the 6 most highly up-regulated and the 2 most highly down-regulated genes and pathology (BC vs normal bladder). The relative mRNA expression examined was normalized to the amount of RPL37A. Statistical significance was determined by the Mann-Whitney U test.

Real-time RT-PCR verification of microarray results. We subjected the 6 most highly up-regulated genes and the 2 most highly down-regulated genes (Tables II and III) to realtime PCR and compared our results with those obtained for normal bladder (n=4) (Fig. 2). We found that the genes shown by microarray analysis as up-regulated did, indeed, show higher expression in BC than the normal bladder $[FABP5: 0.11\pm0.01 \text{ (normal bladder) vs } 0.96\pm0.17 \text{ (BC)},$ p<0.01; PABPC1: 0.24±0.05 (normal bladder) vs 1.87±0.61 (BC), p<0.01; CKS2: 0.1±0.01 (normal bladder) vs 2.1±0.64 (BC), p<0.01; SF3B1: 0.56±0.15 (normal bladder) vs 2.27±0.54 (BC), p=0.02; DDX5: 0.82±0.08 (normal bladder) vs 3.37±0.57 (BC), p<0.01; EIF3S6: 0.19±0.35 (normal bladder) vs 0.89±0.16 (BC), p<0.01; Fig. 2A]. The genes we identified as down-regulated manifested a lower expression in BC than the normal bladder [TAGLN: 0.74±0.21 (normal bladder) vs 0.02±0.02 (BC), p<0.01; TPM2: 0.96±0.22 (normal bladder) vs 0.09±0.03 (BC), p<0.01; Fig. 2B]. Therefore, our microarray results reflect the actual mRNA levels of genes examined in our BC series.

Relationship between up-regulated genes and the BC stage. Using the 6 most highly up-regulated genes in Table II, we compared the mRNA expression level in superficial- and invasive BCs. Real-time PCR of the 14 BCs subjected to microarray analysis demonstrated that *CKS2* was the only gene with significantly higher up-regulation in invasive than in superficial BC (p=0.04) (Fig. 3). There was no difference between superficial- and invasive BC for the other 5 examined genes (Fig. 3). To confirm our results, we subjected all 21 BCs in this series to real-time PCR assay of *CKS2* (Table I). As shown in Fig. 4, there was a high degree of difference between superficial- and invasive BC $[0.79\pm0.15$ (superficial) vs 2.78 ± 0.52 (invasive), p=0.001] and between the normal bladder and invasive BC $[0.12\pm0.01$ (normal bladder) vs 2.78 ± 0.52 (invasive BC), p=0.001]; there was less difference between the normal bladder and superficial BC (p=0.005).

Discussion

We attempted to identify novel biomarkers for human BC by gene expression analysis of oligoarrays. We found that all 14 BCs subjected to microarray analysis shared 21 up- and 25 down-regulated genes; real-time RT-PCR analysis of the 6 most highly up-regulated and the 2 most highly downregulated genes confirmed our microarray results, indicating that they indeed reflect the actual mRNA levels of the genes examined in our BC series. Like others (13,18), we used our microarray results to classify up- and down-regulated genes by their function. We found that 36% were involved in metabolism and 14% in transcription and processing. This may implicate them in the development and progression of BC (Fig. 1). The major function of our down-regulated genes was cell adhesion/surface- (25%) or cytoskeleton/cell membrane-related (21%), suggesting that BC cells acquire the ability to migrate via the down-regulation of these genes.

Assessment of the location of the 136 up-regulated genes showed that 11 (8.1%) were on chromosome 7p, 10 (7.4%) on 8q, 8 (5.9%) on 12q, 7 each (5.1%) on 1p, 1q, and 2q, 6 each (4.4%) on 15q and 17q, 5 each (3.7%) on 2p and 20q,

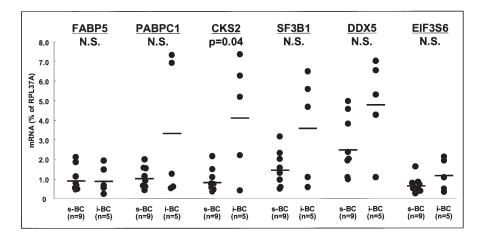


Figure 3. Comparison of the real-time PCR results for the 6 most highly up-regulated genes and the BC stage. Using samples from the 14 BCs subjected to microarray analysis, we found that *CKS2* was the only gene that was significantly up-regulated in invasive compared to superficial BC. The relative mRNA expression examined was normalized to the amount of RPL37A. Statistical significance was determined by the Mann-Whitney U test.

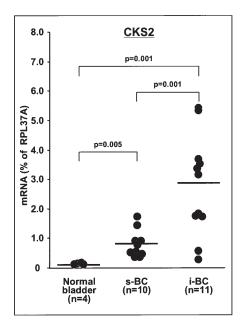


Figure 4. Real-time PCR analysis of *CKS2* expression in tissue from normal bladders and 21 bladders with superficial or invasive cancer. There was a significant difference between superficial and invasive BC (p=0.001), between normal bladders and bladders with invasive BC (p=0.001), and between normal bladders and bladders with superficial BC (p=0.005). The relative mRNA expression examined was normalized to the amount of RPL37A. Statistical significance was determined by the Bonferroni-adjusted Mann-Whitney U test.

and 4 each (2.9%) on 5q, 7q, 14q, and 22q (Table II). Others (19-21) have shown by comparative genomic hybridization (CGH) that the development and progression of BC is characterized by specific amplification involving chromosomes 1q, 2p, 3q, 5p, 6p, 8q, 11q, 17q, and 20q. We posit that several of the up-regulated genes in our BC series are associated with specific chromosomal amplification important in the development and progression of BC.

Among the 6 most highly up-regulated genes in our microarray analysis, *FABP5*, the gene with the highest level of up-regulation, carries fatty acids (FA) through the aqueous

cellular environment and is involved in processes such as FA uptake, -transport, and -oxidation (22). This gene was also up-regulated in hepatocellular carcinoma cells compared with normal hepatocytes (23). PABPC1 is involved in translation and in the regulation of mRNA decay (24); it is significantly over-expressed in prostate cancer (25). CKS2, which encodes a cyclin kinase subunit of Cdc28/CDC2, is involved in cell-cycle progression from G1 to S and from G2 to M; it is associated with lymphoid cell proliferation and its expression was increased in human acute lymphoblastic leukemia (26, 27). In addition, CKS2 was expressed at significantly higher levels in colon cancer with than without liver metastasis (26). SF3B1 is absolutely required for premRNA splicing (28); its relationship with human cancer has not been reported. DDX5 (p68) is a prototypic member of the so-called DEAD box family of proteins (29) and an established RNA helicase (30); immunohistochemistry and Western blots showed it to be consistently over-expressed in colon cancer compared with matched normal tissues (31). EIF3S6 was first identified as a common virus insertion site in virally-induced mouse mammary tumors and preneoplastic lesions (32). Buttitta et al (33) reported that early-stage nonsmall cell lung cancer exhibited EIF3S6 mRNA levels higher than those observed in matching normal lung tissues. Ours is the first detailed investigation of these genes in human BC and our results suggest that they may be promising candidates for diagnostic BC biomarkers.

Bladder tumor antigen, the nuclear matrix protein 22, and the urinary bladder cancer antigen are clinically available diagnostic biomarkers for BC (6,7). However, because of their insufficient sensitivity and specificity they cannot replace cystoscopy or cytology (3,7) and patients with suspected BC continue to require painful cystoscopy. Microarray analysis of human BC has identified new cancerrelated genes, e.g. *CKS2* (4,13-15), *NPM1* (11,14), *PMSA* (13), and *PCNA* (4,13,15) and the results of gene profiling disclosed their association with tumor stage and progression and clinical outcomes. Although these studies have yielded useful insights into the molecular biology of human BC, they listed the genes without assessment of their value as biomarkers. We focused on the 6 genes that were most highly up-regulated in our microarray analysis and found that *CKS2* was uniquely and significantly up-regulated not only when we compared BC to normal bladder, but also when comparing invasive to superficial BC. Therefore, the *CKS2* gene may be a biomarker not only for diagnosing but also for staging BC. The difference in the *CKS2* expression level between invasive BC and the normal bladder was greater than between superficial BC and the normal bladder (p<0.001 vs p<0.005, Fig. 4), suggesting that *CKS2* expression may influence BC progression via cell-cycle progression. Studies are underway in our laboratory to elucidate the interactions between *CKS2* and related genes in BC and to assess the role of the down-regulated genes.

In conclusion, using oligonucleotide microarrays, we found that the *CKS2* gene may be a biomarker for the diagnosis and staging of BC. Ours is the first report demonstrating that *CKS2* expression is strongly correlated with the progression of human BC. Our comprehensive expression profiling data of BC provide new insight into the molecular biology of BC.

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