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 up-regulated 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 *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.

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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

Table I. Patient characteristics.

No.	Age	Gender	Stage	Grade
1	55	F	superficial	G2
2	61	M	superficial	G2
3	68	M	superficial	G2
4	68	M	superficial	G2
5	88	M	invasive	G3
6	80	M	invasive	G3
7	53	F	superficial	G2
8	85	M	superficial	G2
9	53	M	superficial	G2
10	78	M	invasive	G3
11	83	M	invasive	G2
12	74	F	superficial	G2
13	89	M	superficial	G3
14	70	M	invasive	G3
15	47	F	superficial	G2
16	71	M	invasive	G3
17	75	M	invasive	G3
18	87	F	invasive	G2
19	76	M	invasive	G3
20	59	M	invasive	G3
21	78	M	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 μg 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 ethanol-precipitated aRNA (5 μg) and 5 μl 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 μg) was mixed with 0.5 μg of oligo-dT primer and 0.4 μl of dNTPs (25 mM); a final volume of 25 μl 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 proliferation
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 ribonucleoprotein a2/b1, isoform b1	NM_031243	Hs.487774	7p15	2.634	100.0%	transcription 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 l37	NM_000997	Hs.80545	5p13	2.328	100.0%	translation 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
26	*KRT8*	keratin 8	NM_002273	Hs.533782	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
32	*TXNL5*	thioredoxin-like 5	BC006405	Hs.408236	17p13.1	3.761	92.9%	metabolism
33	*PSMA5*	proteasome (prosome, macropain) subunit, alpha type, 5	NM_002790	Hs.485246	1p13	3.623	92.9%	metabolism
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
40	*HSPD1*	heat shock 60kd protein 1 (chaperonin)	NM_002156	Hs.567290	2q33.1	3.166	92.9%	metabolism

Table II. Continued.

No.	Symbol	Gene name	Accession	UniGene	Location	Median	% Up	Function
41	*CPNE3*	copine iii	NM_003909	Hs.191219	8q21.3	3.069	92.9%	metabolism
42	*PCNA*	proliferating cell nuclear antigen	NM_002592	Hs.147433	20pter-p12	3.062	92.9%	cell proliferation
43	*EBNA1BP2*	ebna1 binding protein 2	NM_006824	Hs.346868	1p35-p33	3.030	92.9%	translation and processing
44	*HSPC016*	hypothetical protein HSPC016	NM_015933	Hs.356440	3p21.31	2.911	92.9%	EST
45	*GNL3*	guanine nucleotide binding protein-like 3	NM_014366	Hs.313544	3p21.1	2.891	92.9%	cell proliferation
46	*NFE2L2*	nuclear factor (erythroid-derived 2)-like 2	NM_006164	Hs.155396	2q31	2.876	92.9%	transcription and processing
47	*XEDAR*	x-linked ectodysplasin receptor	NM_021783	Hs.302017	Xq12	2.806	92.9%	others
48	*RPL6*	ribosomal protein l6	NM_000970	Hs.546283	12q24.1	2.790	92.9%	signal transduction
49	*COPS5*	cop9 (constitutive photomorphogenic, arabidopsis, homolog) subunit 5	NM_006837	Hs.491912	8q13.2	2.781	92.9%	transcription and processing
50	*ALK*	anaplastic lymphoma kinase (Ki-1)	NM_004304	Hs.196534	2p23	2.765	92.9%	cytoskeleton/ cell membrane
51	*STATIP1*	signal transducer and activator of transcription 3 interacting protein 1	NM_018255	Hs.8739	18q12.2	2.611	92.9%	transcription and processing
52	*CCT8*	chaperonin containing tcp1, subunit 8 (theta)	NM_006585	Hs.125113	21q22.11	2.572	92.9%	metabolism
53	*ATP5J2*	atp synthase, h+ transporting, mitochondrial f0 complex, subunit f, isoform 2	NM_004889	Hs.521056	7q22.1	2.524	92.9%	mitochondrion
54	*ATP5F1*	atp synthase, h+ transporting, mitochondrial f0 complex, subunit b, isoform 1	NM_001688	Hs.514870	1p13.2	2.519	92.9%	mitochondrion
55	*PGK1*	phosphoglycerate kinase 1	NM_000291	Hs.78771	Xq13	2.503	92.9%	metabolism
56	*FLJ22875*	hypothetical protein FLJ22875	NM_032231	Hs.439548	15q22.31	2.431	92.9%	EST
57	*LPIN3*	Homo sapiens lipin 3	NM_022896	Hs.528618	20q11.2-q12	2.428	92.9%	metabolism
58	*SMARCA4*	swi/snf related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	NM_003072	Hs.327527	19p13.2	2.411	92.9%	transcription and processing
59	*PSMA2*	proteasome (prosome, macropain) subunit, alpha type, 2	NM_002787	Hs.333786	7p14.1	2.397	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	*TCEB1*	transcription elongation factor B (SIII), polypeptide 1	NM_005648	Hs.554594	8q21.11	2.132	92.9%	transcription and processing
65	*CRHR2*	corticotropin releasing hormone receptor 2	NM_001883	Hs.546246	7p14.3	2.101	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	*RPL26*	ribosomal protein l26	NM_000987	Hs.528879	17p13	3.456	85.7%	metabolism
70	*CBX3*	heterochromatin-like protein 1	BC000954	Hs.381189	7p15.2	3.419	85.7%	transcription and processing
71	*GA17*	dendritic cell protein; ga17	NM_006360	Hs.502244	11p13	3.417	85.7%	cytoskeleton/ cell membrane
72	*S100A2*	S100 calcium binding protein A2	NM_005978	Hs.516484	1q21	3.285	85.7%	others
73	*TAX1BP1*	tax1 (human t-cell leukemia virus type i) binding protein 1	NM_006024	Hs.34576	7p15	3.284	85.7%	cell proliferation
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	*S100A1*	s100 calcium-binding protein a11 (calgizzarin)	BC001410	Hs.417004	1q21	3.259	85.7%	metabolism
77	*HSPC064*	WD repeats and SOF1 domain containing	NM_015420	Hs.532265	8q22.3	3.229	85.7%	others
78	*CETN3*	centrin 3	NM_004365	Hs.128073	5q14.3	3.141	85.7%	metabolism
79	*PDCD10*	programmed cell death 10	NM_007217	Hs.478150	3q26.1	3.139	85.7%	others
80	*WWOX*	for ii protein	AF227527	Hs.461453	16q23.3-q24.1	2.873	85.7%	metabolism
81	*TRIM33*	tripartite motif-containing 33 protein	NM_015906	Hs.26837	1p13.1	2.850	85.7%	metabolism

Table II. Continued.

No.	Symbol	Gene name	Accession	UniGene	Location	Median	% Up	Function
82	-	ensembl genscan prediction	AL163932	-	chromosome 14	2.846	85.7%	EST
83	*PP*	pyrophosphatase (inorganic)	NM_021129	Hs.437403	10q11.1-q24	2.837	85.7%	metabolism
84	*CYCS*	cytochrome c	NM_018947	Hs.437060	7p15.3	2.792	85.7%	mitochondrion
85	*FXYD3*	fxyd domain-containing ion transport regulator 3	BT006712	Hs.301350	19q13.11-q13.12	2.769	85.7%	metabolism
86	*USP47*	ubiquitin specific peptidase 47	NM_017944	Hs.567521	11p15.3	2.760	85.7%	metabolism
87	*RAB11A*	rab11a, member ras oncogene family	NM_004663	Hs.321541	15q21.3-q22.31	2.750	85.7%	signal transduction
88	*PSMD14*	26s proteasome-associated pad1 homolog	NM_005805	Hs.567410	2q24.2	2.724	85.7%	metabolism
89	*C13orf12*	chromosome 13 open reading frame 12	NM_015932	Hs.268742	13q12.3	2.698	85.7%	others
90	*RB1*	retinoblastoma 1	NM_000321	Hs.408528	13q14.2	2.691	85.7%	cell proliferation
91	*ATP2B1*	atpase, ca^{++} transporting, plasma membrane 1	NM_001682	Hs.506276	12q21.3	2.690	85.7%	metabolism
92	*LOC554202*	hypothetical LOC554202	BC011715	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%	translation and processing
99	-	ensembl genscan prediction	AL031186	-	22q12.1-12.2	2.565	85.7%	EST
100	*SRI*	sorcin	NM_003130	Hs.489040	7q21.1	2.552	85.7%	metabolism
101	*FLJ22662*	hypothetical protein flj22662	NM_024829	Hs.131933	12p13.1	2.541	85.7%	EST
102	*RAN*	ras-related nuclear protein	NM_006325	Hs.10842	12q24.3	2.534	85.7%	cell proliferation
103	*DC6*	dc6 protein	NM_020189	Hs.492555	8q23.1	2.531	85.7%	transcription and processing
104	*VPS35*	vacuolar protein sorting 35 (yeast)	NM_018206	Hs.454528	16q12	2.519	85.7%	metabolism
105	-	ensembl genscan prediction	AC080053	-	chromosome 11	2.512	85.7%	EST
106	*KIAA1542*	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%	intracellular organelle
108	*SCFD1*	sec1 family domain containing 1	NM_016106	Hs.369168	14q12	2.479	85.7%	metabolism
109	*PIGL*	phosphatidylinositol glycan, class l	NM_004278	Hs.499793	17p12-p11.2	2.465	85.7%	metabolism
110	*LSM3*	LSM3 homolog, U6 small nuclear RNA associated	NM_014463	Hs.111632	3p25.1	2.454	85.7%	transcription and processing
111	*WDR61*	WD repeat domain 61	AF309553	Hs.513055	15q25.1	2.451	85.7%	transcription and processing
112	*LOC389651*	similar to hypothetical protein (L1H 3 region)	XM_372039	Hs.567978	8p11.1	2.434	85.7%	EST
113	*LEPRE1*	growth suppressor 1	NM_022356	Hs.437656	1p34.1	2.424	85.7%	others
114	-	ensembl genscan prediction	AC011371	-	chromosome 5	2.402	85.7%	EST
115	*GNGT2*	guanine nucleotide binding protein-gamma transducing activity polypeptide 2	NM_031498	Hs.181781	17q21	2.393	85.7%	signal transduction
116	*NRBF2*	nuclear receptor binding factor 2	NM_030759	Hs.449628	10q21.3	2.388	85.7%	transcription and processing
117	*ARPC2*	actin related protein 2/3 complex, subunit 2	NM_005731	Hs.529303	2q36.1	2.380	85.7%	cytoskeleton/ cell membrane
118	*SEC61G*	sec61 gamma	NM_014302	Hs.488282	7p11.2	2.378	85.7%	intracellular organelle
119	*SSBP1*	single-stranded dna-binding protein	NM_003143	Hs.490394	7q34	2.369	85.7%	mitochondrion
120	*C14orf112*	chromosome 14 open reading frame 112	NM_016468	Hs.137108	14q24.2	2.367	85.7%	others
121	*ATP5E*	atp synthase, h+ transporting, mitochondrial f1 complex, epsilon subunit	NM_006886	Hs.177530	20q13.32	2.362	85.7%	mitochondrion
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
125	*PPIA*	peptidylprolyl isomerase a (cyclophilin a)	NM_021130	Hs.356331	7p13-p11.2	2.319	85.7%	metabolism

Table II. Continued.

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

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 down-regulated genes is presented in Fig. 1. Functionally, the up-regulated genes were involved in metabolism (36%), transcription 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).

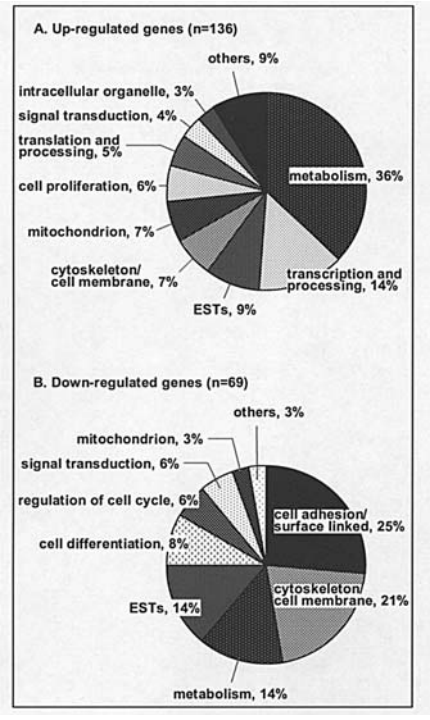

Figure 1. Distribution of the expression of functionally categorized genes in human BC. The functional features of the 136 up- (A) and 69 down-regulated genes (B) were classified into 13 categories.

Table III. Frequently down-regulated genes in bladder cancer.

No.	Symbol	Gene name	Accession	UniGene	Location	Median	% Up Down	Function
1	*TAGLN*	transgelin	NM_003186	Hs.503998	11q23.2	0.095	100.0%	cytoskeleton/ cell membrane
2	*TPM2*	tropomyosin 2 (beta)	NM_003289	Hs.300772	9p13.2-p13.1	0.113	100.0%	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	100.0%	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	0.247	92.9%	metabolism
31	*COL3A1*	alpha 1 type iii collagen preproprotein	NM_000090	Hs.443625	2q31	0.266	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 Down	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/ cell membrane
36	*PDLIM3*	alpha-actinin-2-associated lim protein	NM_014476	Hs.85862	4q35	0.317	92.9%	cytoskeleton/ cell membrane
37	*AEBP1*	adipocyte enhancer binding protein 1 precursor	NM_001129	Hs.439463	7p13	0.350	92.9%	cell adhesion/ 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/ cell membrane
42	*PTGDS*	prostaglandin d2 synthase (21kd, brain)	NM_000954	Hs.446429	9q34.2-q34.3	0.418	92.9%	metabolism
43	*PRSS23*	putative secreted protein zsig13	AF193611	Hs.25338	11q14.1	0.421	92.9%	EST
44	*CKIP-1*	ck2 interacting protein 1; hq0024c protein; loc51177	NM_016274	Hs.438824	1q21.2	0.435	92.9%	EST
45	*TIMP1*	tissue inhibitor of metalloproteinase 1 precursor	NM_003254	Hs.522632	Xp11.3-p11.23	0.453	92.9%	cell adhesion/ 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 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/ 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, clade f member 1	NM_002615	Hs.532768	17p13.1	0.409	85.7%	cell differentiation
56	-	nc_001807 mitochondrion complete genome	NC_001807	-	mitochondrion	0.414	85.7%	mitochondrion
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 (muscle)	NM_001864	Hs.421621	19q13.1	0.431	85.7%	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	-	ensembl genscan prediction	AF277315	-	Xq28	0.484	85.7%	EST
65	*SPARC*	secreted protein, acidic, cysteine-rich (osteonectin)	NM_003118	Hs.111779	5q31.3-q32	0.484	85.7%	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%	cell 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

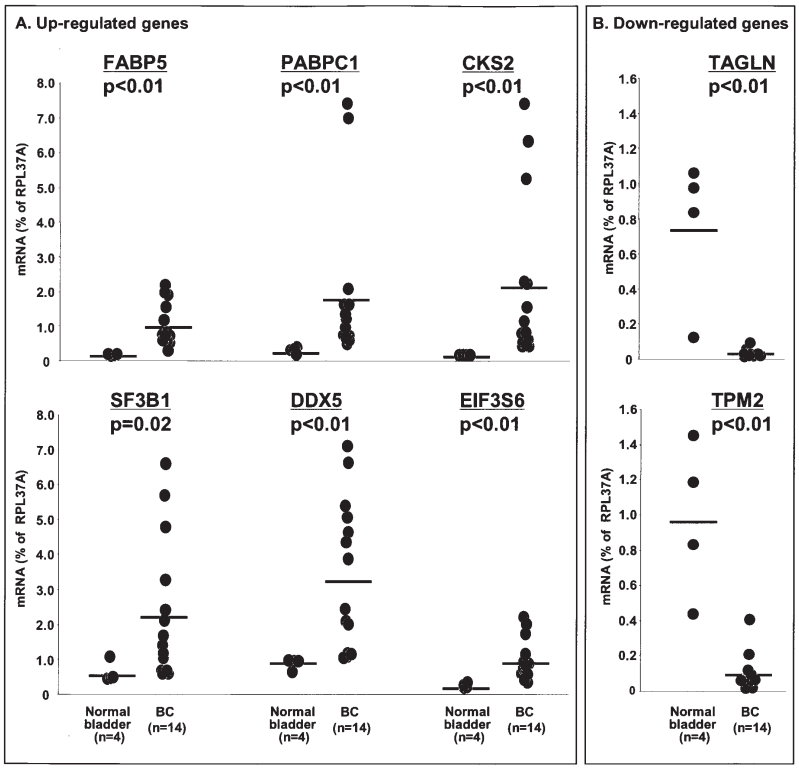

Figure 2. Comparison of the real-time PCR results for the 6 most highly up-regulated and the 2 most highly down-regulated genes and pathology (BC vs normal bladder). The relative mRNA expression examined was normalized to the amount of RPL37A. Statistical significance was determined by the Mann-Whitney 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 real-time 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±0.01 (normal bladder) vs 0.96±0.17 (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±0.15 (superficial) vs 2.78±0.52 (invasive), p=0.001] and between the normal bladder and invasive BC [0.12±0.01 (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 down-regulated 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,

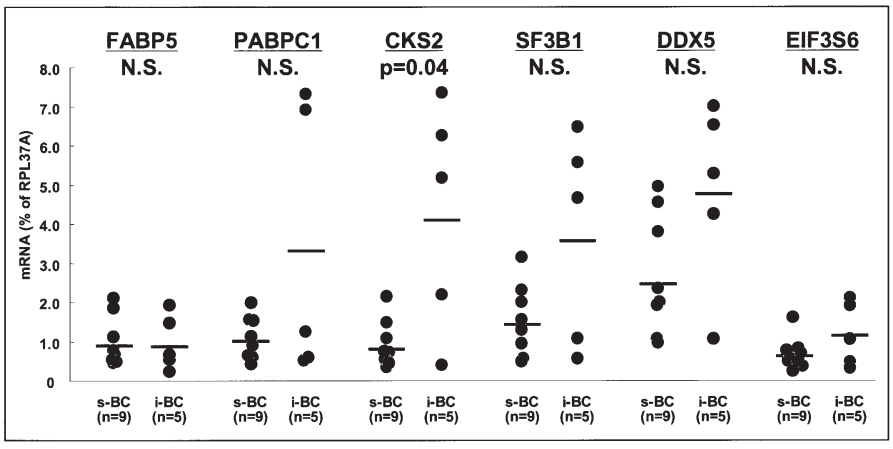

Figure 3. Comparison of the real-time PCR results for the 6 most highly up-regulated genes and the BC stage. Using samples from the 14 BCs subjected to microarray analysis, we found that *CKS2* was the only gene that was significantly up-regulated in invasive compared to superficial BC. The relative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

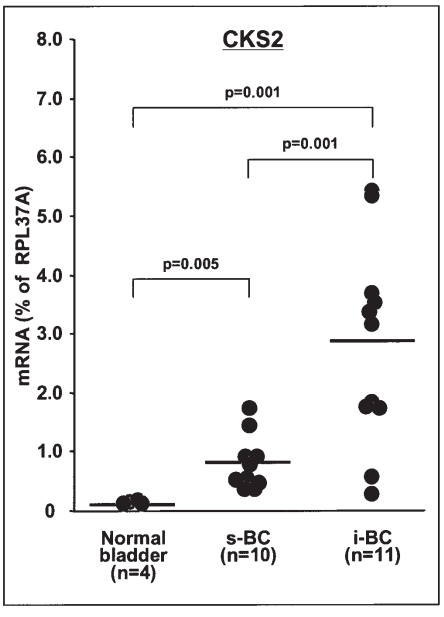

Figure 4. Real-time PCR analysis of *CKS2* expression in tissue from normal bladders and 21 bladders with superficial or invasive cancer. There was a significant difference between superficial and invasive BC (p=0.001), between normal bladders and bladders with invasive BC (p=0.001), and between normal 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 pre-mRNA 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 non-small 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 cancer-related 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.

Acknowledgements

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