Table 1

Spot no.[a]	Access. no.[b]	Swiss-prot name[c]	Protien description[d]	Theoretical[e] (Mr/p*I*)	Experimental[f] (Mr/p*I*)	Sequence[g] coverage (%)
Nucleic acid binding						
20	Q07244	ROK_HUMAN	Heterogeneous nuclear ribonucleoprotein K (hnRNP K)	51.3/5.28	60.5/5.29	32.1
23	Q07244	ROK_HUMAN	Heterogeneous nuclear ribonucleoprotein K (hnRNP K)	51.3/5.28	61.1/5.58	33.5
71	P08865	RSP4_HUMAN	40S ribosomal protein SA (P40)	33.0/4.62	43.7/4.72	50.8
78	P55795	ROH2_HUMAN	Heterogeneous nuclear ribonucleoprotein H′ (HNRNP H′)	49.5/6.26	53.4/6.21	30.5
86	P26641	EF1G_HUMAN	Elongation factor 1-gamma (EF-1-gamma)	50.5/6.64	50.2/6.32	43.2
87	P38919	IF4N	Eukaryotic initiation factor 4A-like NUK-34	47.1/6.4	50.5/6.47	11.9
90	P49591	SYS_HUMAN	Seryl-tRNA synthetase (serine-tRNA ligase)	59.3/6.39	59.2/6.46	28.6
94	P13639	EF2_HUMAN	Elongation factor 2 (EF-2)	96.2/6.8	96.6/6.92	38.6
95	P13639	EF2_HUMAN	Elongation factor 2 (EF-2)	96.2/6.8	96.1/7.12	43.5
97	P14866	ROL_HUMAN	Heterogeneous nuclear ribonucleoprotein L (hnRNP L)	60.8/7.1	63.2/7.22	9.4
103	P49411	EFTU_HUMAN	Elongation factor Tu, mitochondrial precursor (P43)	49.9/7.6	48.2/6.80	11.5
114	P04720	EF11_HUMAN	Elongation factor 1-alpha 1 (EF-1-alpha-1)	50.5/9.71	58.5/9.02	11.5
126	P22626	ROA2_HUMAN	Heterogeneous nuclear ribonucleoproteins A2/B1	37.5/9.77	34.8/8.65	18.1
127	P22626	ROA2_HUMAN	Heterogeneous nuclear ribonucleoproteins A2/B1	37.5/9.77	34.6/8.87	24.4
135	Q15365	PCB1_HUMAN	Poly (rC)-binding protein 1 (Alpha-CP1)	38.0/7.17	42.1/7.03	46.9
141	P05388	RLA0_HUMAN	60S Acidic ribosomal protein P0 (L10E)	34.4/5.83	35.9/6.59	28.7
145	P05198	IF2A_HUMAN	Eukaryotic translation initiation factor 2 subunit 1 (EIF-2A)	36.4/4.84	39.3/5.27	35.2
148	Q13347	IF32_HUMAN	Eukaryotic translation initiation factor 3 subunit 2	36.9/5.44	38.1/5.64	35.4
149	Q13347	IF32_HUMAN	Eukaryotic translation initiation factor 3 subunit 2	36.9/5.44	38.5/5.75	35.4
168	P54727	R23B_HUMAN	UV excision repair protein RAD23 homolog B (HHR23B)	43.2/4.6	53.2/4.77	18.1
170	P29692	EF1D_HUMAN	Elongation factor 1-delta (EF-1-delta)	31.3/4.97	36.4/4.89	25.6
171	P29692	EF1D_HUMAN	Elongation factor 1-delta (EF-1-delta)	31.3/4.97	36.4/4.97	17.1
173	P12004	PCNA_HUMAN	Proliferating cell nuclear antigen (PCNA) (Cyclin)	29.1/4.4	34.8/4.45	28.0
194	Q13347	IF32_HUMAN	Eukaryotic translation initiation factor 3 subunit 2	36.9/5.44	29.4/5.76	46.8
229	Q15056	IF4H_HUMAN	Eukaryotic translation initiation factor 4H (eIF-4H)	27.4/6.67	32.4/6.52	31.0
239	Q07955	SFR1_HUMAN	Splicing factor, arginine/serine-rich 1	27.9/6.48	29.9/7.92	45.6
266	P24534	EF1B_HUMAN	Elongation factor 1-beta (EF-1-beta)	24.8/4.33	32.1/4.14	63.8
270	Q15056	IF4H_HUMAN	Eukaryotic translation initiation factor 4H (eIF-4H)	27.4/6.67	31.5/6.45	8.6
281	O00571	DDX3_HUMAN	DEAD-box protein 3 (Helicase-like protein 2)	73.5/7.17	80.3/6.77	34.3
286	O00571	DDX3_HUMAN	DEAD-box protein 3 (Helicase-like protein 2)	73.5/7.17	84.6/7.14	34.3
298	P12956	KU70_HUMAN	ATP-dependent DNA helicase II, 70 kDa subunit (Ku70)	70.0/6.61	73.3/6.61	37.3
299	P11940	PAB1_HUMAN	Polyadenylate-binding protein 1	70.9/10.31	79.6/8.29	18.4
313	Q99880	H2BC_HUMAN	Histone H2B.c (H2B/c)	13.8/11.13	13.8/7.67	75.2
Oxidoreductase						
21	P07237	PDI_HUMAN	Protein disulfide isomerase precursor (PDI)	57.5/4.59	60.5/5.29	32.1
33	P48163	MAOX_HUMAN	NADP-dependent malic enzyme (NADP-ME)	64.7/6.04	63.1/5.94	10.1
46	P31040	DHSA_HUMAN	Succinate dehydrogenase (ubiquinone) flavoprotein subunit	73.7/7.34	70.0/6.59	27.1
74	P31930	UCR1_HUMAN	Ubiquinol-cytochrome C reductase complex core protein I	53.3/6.33	49.8/5.67	16
80	P30837	DHA5_HUMAN	Aldehyde dehydrogenase X, mitochondrial precursor (ALDH class 2)	57.7/6.83	54.7/6.28	30.4
91	O43175	SERA_HUMAN	D-3-Phosphoglycerate dehydrogenase (PGDH)	57.4/6.68	56.8/6.69	7.5
98	O60701	UGDH_HUMAN	UDP-glucose 6-dehydrogenase (UDP-glc dehydrogenase)	55.7/7.08	58.9/7.23	20.4
100	P00367	DHE3_HUMAN	Glutamate dehydrogenase 1, mitochondrial precursor (GDH)	61.7/7.82	54.1/7.17	12.2
101	P11413	G6PD_HUMAN	Glucose-6-phosphate 1-dehydrogenase (G6PD)	59.7/6.87	59.1/6.99	31.3
104	O75874	IDHC_HUMAN	Isocit rate dehydrogenase (NADP) cytoplasmic	47.0/6.79	46.8/6.88	19.1
105	O75874	IDHC_HUMAN	Isocit rate dehydrogenase (NADP) cytoplasmic	47.0/6.79	46.7/6.91	22.0
122	P00338	LDHA_HUMAN	L-lactate dehydrogenase A chain (LDH-A)	37.0/8.34	35.2/8.42	66.3
123	P04406	G3P2_HUMAN	Glyceraldehyde 3-phosphate dehydrogenase, liver	36.2/8.73	36.4/8.06	43.3
124	P04406	G3P2_HUMAN	Glyceraldehyde 3-phosphate dehydrogenase, liver	36.2/8.73	36.2/8.74	43.3
125	P04406	G3P2_HUMAN	Glyceraldehyde 3-phosphate dehydrogenase, liver	36.2/8.73	36.2/8.74	43.3
136	P36551	HEM6_HUMAN	Coproporphyrinogen III oxidase, mitochondrial precursor	40.9/7.11	38.3/7.24	46.9
140	P40925	MDHC_HUMAN	Malate dehydrogenase, cytoplasmic	36.7/7.37	35.6/7.04	21.0
141	P40925	MDHC_HUMAN	Malate dehydrogenase 1, NAD (soluble)	36.4/6.91	35.9/6.59	43.5
144	P14550	ALDX_HUMAN	Alcohol dehydrogenase [NADP+] (Aldehyde reductase)	36.9/6.78	38.8/6.86	33.5
159	O75874	IDHC_HUMAN	Isocitrate dehydrogenase (NADP) cytoplasmic	47.0/6.79	39.2/5.92	52.7
159	O75874	IDHC_HUMAN	Isocitrate dehydrogenase 3 (NAD+) alpha	47.0/6.79	39.2/5.92	20.0
162	P07195	LDHB_HUMAN	L-lactate dehydrogenase B chain (LDH-B)	36.9/5.96	36.7/5.84	31.7
162	P07195	LDHB_HUMAN	L-lactate dehydrogenase B chain (LDH-B	36.9/5.96	36.7/5.84	40.7
203	P32119	PDX2_HUMAN	Peroxiredoxin 2 (Thioredoxinperoxidase 1)	22.1/5.8	26.4/5.61	44.9
210	P30041	AOP2_HUMAN	Antioxidant protein 2 (1-cis peroxiredoxin)	25.1/6.31	29.3/6.47	69.2
212	P30048	PDX3_HUMAN	Peroxiredoxin 3; antioxidant protein 1	27.7/7.67	27.4/6.16	5.0
230	P13804	ETFA_HUMAN	Electron transfer flavoprotein alpha-subunit	35.4/8.44	33.4/7.04	23.4

Table 1 (*Continued*)

Spot no.[a]	Access. no.[b]	Swiss-prot name[c]	Protien description[d]	Theoretical[e] (Mr/p*I*)	Experimental[f] (Mr/p*I*)	Sequence[g] coverage (%)
231	P13804	ETFA_HUMAN	Electron transfer flavoprotein alpha-subunit	35.4/8.44	33.5/7.27	23.4
232	P13804	ETFA_HUMAN	Electron transfer flavoprotein alpha-subunit	35.4/8.44	33.4/7.47	23.4
235	P31930	UCR1_HUMAN	Ubiquinol-cytochrome C reductase complex core protein I	53.3/6.33	29.0/7.05	24.1
249	Q99714	HCD2_HUMAN	3-Hydroxyacyl-CoA dehydrogenase type II (Type II HADH)	27.2/7.79	28.3/7.36	26.1
253	Q06830	PDX1_HUMAN	Peroxiredoxin 1 (Thioredoxin peroxidase 2)	22.3/8.18	26.3/8.33	41.2
254	P30044	PDX5_HUMAN	Peroxiredoxin 5, mitochondrial	22.1/8.85	16.3/7.40	5.6
267	O43396	TXNL_HUMAN	Thioredoxin-like protein (32 kDa thioredoxin-related protein)	32.7/4.67	35.8/5.01	38.8
271	P31930	UCR1_HUMAN	Ubiquinol-cytochrome C reductase complex core protein I	53.3/6.33	29.3/6.47	24.1
273	Q06830	PDX1_HUMAN	Peroxiredoxin 1 (Thioredoxin peroxidase 2)	22.3/8.18	26.2/7.70	39.2
283	P28331	NUAM_HUMAN	NADH-ubiquinone oxidoreductase 75 kDa	80.6/5.98	88.1/7.13	23.9
304	O60701	UGDH_HUMAN	UDP-glucose 6-dehydrogenase (UDPGDH)	55.7/7.08	57.9/7.11	35.8
307	P13804	ETFA_HUMAN	Electron transfer flavoprotein alpha-subunit	35.4/8.44	33.5/7.27	35.4
Cytoskeletal protein						
5	O43707	AAC4_HUMAN	Alpha-actinin 4 (F-actin cross linking protein)	105.3/5.21	99.1/5.4	7.1
16	P20700	LAM1_HUMAN	Lamin B1	66.7/4.94	70.4/5.27	54.1
34	P04264	K2C1_HUMAN	Keratin, type II cytoskeletal 1 (CK 1)	66.2/8.33	68.1/6.01	13.8
37	P06396	GELS_HUMAN	Gelsolin precursor, plasma	86.1/5.90	88.6/5.9	13.6
39	P15311	EZRI_HUMAN	Ezrin (p81) (cytovillin) (villin2)	69.5/6.2	81.1/6.2	29.0
40	P15311	EZRI_HUMAN	Ezrin (p81) (cytovillin) (villin2)	69.5/6.2	81.8/6.38	33.4
42	P35221	CTN1_HUMAN	Alpha-1 catenin	100.8/6.2	98.8/6.16	14.3
43	P18206	VINC_HUMAN	Vinculin (metavinculin)	117.3/8	110.1/6.21	8.4
44	P18206	VINC_HUMAN	Vinculin (metavinculin)	117.3/8	110.1/6.26	8.4
52	P35900	K1CT_HUMAN	Keratin, type I cytoskeletal 20	48.5/5.52	50.6/5.64	3.1
57	P05787	K2C8_HUMAN	Keratin, typeII cytoskeletal 8 (CK 8)	53.7/5.37	55.9/5.70	91.6
58	P05217	TBB2_HUMAN	Tubulin beta-2 chain	50.3/4.63	53.1/4.97	52.4
59	P05209	TBA1_HUMAN	Tubulin alpha-1 chain, brain-specific	50.8/4.89	55.4/5.16	45.7
65	P05787	K2C8_HUMAN	Keratin, typeII cytoskeletal 8 (CK 8)	53.7/5.37	53.7/5.57	42.8
66	P05783	K1CR_HUMAN	Keratin, type I cytoskeletal 18 (CK 18)	48.5/5.22	47.4/5.50	77.4
67	P02570	ACTB_HUMAN	Actin, cytoplasmic 1 (beta-actin)	42.1/5.24	45.3/5.24	48.0
68	P02570	ACTB_HUMAN	Actin, cytoplasmic 1 (beta-actin)	42.1/5.42	46.0/5.14	50.3
69	P08727	K1CS_HUMAN	Keratin, type I cytoskeletal 19 (CK 19)	44.1/4.89	43.6/5.07	27.5
70	P08727	K1CS_HUMAN	Keratin, type I cytoskeletal 19 (CK 19)	44.1/4.89	44.1/4.06	90.3
71	P08727	K1CS_HUMAN	Keratin, type I cytoskeletal 19 (CK 19)	44.1/4.89	43.7/4.72	90.3
73	P32391	ARP3_HUMAN	Actin-like protein 3 (Actin-related protein 3)	47.8/5.74	48.4/5.87	30.1
76	P02570	ACTB_HUMAN	Actin, cytoplasmic 1 (beta-actin)	42.1/5.24	43.7/5.34	25.6
77	P05783	K1CR_HUMAN	Keratin, type I cytoskeletal 18 (CK 18)	48.5/5.22	45.2/5.35	20.2
109	O75083	WDR1_HUMAN	WD-repeated protein 1	66.9/6.64	68.8/7.74	5.9
154	Q15019	SEP2_HUMAN	Septin 2	41.7/6.58	45.3/6.43	32.1
161	P52907	CAZ1_HUMAN	F-actin capping protein alpha-1 subunit	33.1/5.5	35.9/5.57	52.4
174	P12324	TPMN_HUMAN	Tropomyosin, cytoskeletal type	29.3/4.56	33.2/4.42	39.5
175	P12324	TPMN_HUMAN	Tropomyosin, cytoskeletal type	29.3/4.59	32.9/4.58	52.4
181	P10469	TPMS_HUMAN	Tropomyosin alpha chain, smooth muscle	26.6/4.59	31.1/4.58	32.2
188	P47756	CAPB_HUMAN	F-actin capping protein beta subunit	31.0/5.82	33.0/5.72	59.9
275	P007737	PRO1_HUMAN	Profilin I	15.1/8.35	11.6/8.46	64.0
277	Q14247	SRC8_HUMAN	Src substrate cortactin	61.8/5.14	81.3/5.43	54.0
280	P02545	LAMA_HUMAN	Lamin A/C (70 kDa lamin)	74.4/7.0	85.3/6.97	32.8
284	P02545	LAMA_HUMAN	Lamin A/C (70 kDa lamin)	74.4/7.0	81.7/6.92	54.7
301	Q03252	LAM2_HUMAN	Lamin B2	66.9/6.03	70.8/5.46	69.1
Chaperone						
1	Q9Y4L1	OXRP_HUMAN	150 kDa oxygen-regulated protein precursor	111.6/5.0	119.6/5.26	9.1
2	P14625	ENPL_HUMAN	Endoplasmin precursor (GRP94)	92.8/4.58	98.6/4.79	6.0
7	P07900	HS9A_HUMAN	Heat shock protein HSP 90-alpha (HSP 86)	85.1/4.77	87.2/5.02	10.0
8	P11021	GR78_HUMAN	78 kD glucose regulated protein precursor (GRP 78)	72.2/4.86	76.2/5.04	48.1
11	P38646	GR75_HUMAN	Mitochondrial stress-70 protein precursor (GRP 75)	74.1/6.21	72.8/5.61	29.9
12	P08107	HS71_HUMAN	Heat shock 70 kDa protein 1 (HSP70.1)	70.3/5.4	69.1/5.58	31.4
15	P11142	HS7C_HUMAN	Heat shock cognate 71 kDa protein	71.1/5.25	73.0/5.19	71.1
22	P10809	CH60_HUMAN	60 kDa heat shock protein, mitochondrial precursor (Hsp60)	61.2/5.46	58.5/5.37	46.4
24	P48643	TCPE_HUMAN	T-complex protein 1, epsilon subunit (TCP-1-epsilon)	60.1/5.42	58.7/5.66	31.6
25	P54578	UBPE_HUMAN	Ubiquitin carboxyl-terminal hydrolase 14	55.9/5.20	59.3/5.42	4.9
26	P50990	TCPQ_HUMAN	T-complex protein 1, theta subunit	59.6/5.20	58.5/5.58	22.3
27	P14625	ENPL_HUMAN	Endoplasmin precursor (GRP94)	92.8/4.58	99.1/5.89	4.5
29	P50990	TCPQ_HUMAN	T-complex protein 1, theta subunit (TCP-1-theta)	60.2/5.39	58.7/5.66	22.3

Table 1 (*Continued*)

Spot no.[a]	Access. no.[b]	Swiss-prot name[c]	Protien description[d]	Theoretical[e] (Mr/p*I*)	Experimental[f] (Mr/p*I*)	Sequence[g] coverage (%)
32	P11142	HS7C_HUMAN	Heat shock cognate 71 kDa protein	71.1/5.25	66.2/5.79	71.1
36	P07900	HS9A_HUMAN	Heat shock protein HSP 90-alpha (HSP 86)	85.1/4.77	87.9/5.81	10.0
38	P14625	ENPL_HUMAN	Endoplasmin precursor (GRP94)	92.8/4.58	98.6/5.94	31.0
47	P49368	TCPG_HUMAN	T-complex protein 1, gamma subunit (TCP-1-gamma)	60.9/6.6	64.9/6.36	41.2
51	P17987	TCPA_HUMAN	T-complex protein 1, alpha subunit (TCP-1-alpha)	60.9/6.0	61.8/6.05	23.4
54	P78371	TCPB_HUMAN	T-complex protein 1, beta subunit (TCP-1-beta)	57.8/6.42	55.7/5.87	40.4
56	P78371	TCPB_HUMAN	T-complex protein 1, beta subunit (TCP-1-beta)	57.8/6.42	53.3/5.83	26.4
93	P31948	IEFS_HUMAN	Stress-induced-phosphoprotein 1 (STI1)	63.3/6.77	84.3/6.73	54.1
107	5453607	TCPH_HUMAN	Chaperonin containing TCP1, subunit 7 (eta)	59.4/7.55	57.7/7.60	10.0
209	P04792	HS27_HUMAN	Heat shock protein 27 kDa protein (HSP 27)	22.4/8.14	29.4/6.14	54.3
279	P08238	HS9B_HUMAN	Heat shock protein HSP 90-beta (HSP 84) (HSP 90)	83.5/4.79	94.4/6.81	49.8
282	P07900	HS9A_HUMAN	Heat shock protein HSP 90-alpha (HSP 86)	84.9/4.77	88.4/7.04	10.0
314	Q04984	CH10_HUMAN	10 kDa heat shock protein, mitochondrial (Hsp10)	10.8/9.73	9.2/8.98	56.4
Miscellaneous function						
16	P20700	LAM1_HUMAN	Lamin B1	66.7/4.94	70.4/5.27	54.1
57	P05787	K2C8_HUMAN	Keratin, typeII cytoskeletal 8 (CK 8)	53.7/5.37	55.9/5.70	71.6
65	P05787	K2C8_HUMAN	Keratin, typeII cytoskeletal 8 (CK 8)	53.67/5.37	53.7/5.57	42.8
66	P05783	K1CR_HUMAN	Keratin, type I cytoskeletal 18 (CK 18)	48.5/5.22	47.4/5.50	77.4
69	P08727	K1CS_HUMAN	Keratin, type I cytoskeletal 19 (CK 19)	44.1/4.89	43.6/5.07	27.5
70	P08727	K1CS_HUMAN	Keratin, type I cytoskeletal 19 (CK 19)	44.1/4.89	44.1/4.06	90.3
71	P08727	K1CS_HUMAN	Keratin, type I cytoskeletal 19 (CK 19)	44.1/4.89	43.7/4.72	90.3
77	P05783	K1CR_HUMAN	Keratin, type I cytoskeletal 18 (CK 18)	48.5/5.22	45.2/5.35	20.2
164	Q9Y3F4	UNRI_HUMAN	UNR-interacting protein (WD-40 repeat protein PT-WD)	38.8/4.85	41.2/5.05	32.9
177	P42655	143E_HUMAN	14-3-3 protein epsilon	29.3/4.46	32.1/4.41	55.7
188	P47756	CAPB_HUMAN	F-actin capping protein beta subunit	31.0/5.82	33.0/5.72	59.9
193	P42168	IGUP_HUMAN	Interferon gamma up-regulated I-5111 protein	28.9/5.9	31.3/5.84	68.7
195	P52566	GDIS_HUMAN	Rho GDP-dissociation inhibitor 2 (Rho GDI 2)	23.0/4.94	28.0/5.63	19.9
224	P16949	STN1_HUMAN	Stathmin	17.3/5.76	19.8/5.81	11.0
237	P25388	GBLP_HUMAN	Guanine nucleotide-binding protein beta subunit-like protein 12.3	35.5/7.64	32.7/7.60	20.8
268	Q15691	MAE1_HUMAN	Microtubule-associated protein RP/EB family member 1	30.2/4.86	33.3/5.17	26.9
280	P02545	LAMA_HUMAN	Lamin A/C (70 kDa lamin)	74.4/7.0	85.3/6.97	32.8
284	P02545	LAMA_HUMAN	Lamin A/C (70 kDa lamin)	74.4/7.0	81.7/6.92	54.7
Isomerase						
18	P07237	PDI_HUMAN	Protein disulfide isomerase precursor (PDI)	57.5/4.59	59.2/4.77	32.7
18	P07237	PDI_HUMAN	Protein disulfide isomerase precursor (PDI)	57.5/4.59	59.2/4.77	32.7
21	P07237	PDI_HUMAN	Protein disulfide isomerase precursor (PDI)	57.5/4.59	60.5/5.29	32.7
28	P30101	PDA3_HUMAN	Protein disulfide isomerase A3 precursor	57.2/6.29	56.6/5.5	35.6
28	Q02790	FKB4_HUMAN	P59 protein (HSP binding immunophilin)	52.1/5.24	56.6/5.5	17.9
28	P30101	PDA3_HUMAN	Protein disulfide isomerase A3 precursor	57.2/6.29	56.6/5.5	35.6
30	P30101	PDA3_HUMAN	Protein disulfide isomerase A3 precursor	57.2/6.29	57.1/5.77	54.1
30	P30101	PDA3_HUMAN	Protein disulfide isomerase A3 precursor	57.2/6.29	57.1/5.77	54.1
31	P30101	PDA3_HUMAN	Protein disulfide isomerase A3 precursor	57.2/6.29	50.8/5.87	35.6
31	P30101	PDA3_HUMAN	Protein disulfide isomerase A3 precursor	57.2/6.29	50.8/5.87	35.6
50	O95394	AGM1_HUMAN	Phosphoacetylglucosamine mutase (PAGM)	60.3/6.22	64.7/6.11	20.3
133	Q08752	CYP4_HUMAN	40 kDa peptidyl-prolyl cis-trans isomerase	41.2/7.19	42.2/7.18	21.1
234	P18669	PMG1_HUMAN	Phosphoglycerate mutase, muscle form (PGAM-B)	28.9/7.2	31.2/7.10	30.3
234	P15259	PMGM_HUMAN	Phosphoglycerate mutase, muscle form (PGAM-M)	28.9/9.5	30.1/7.01	22.1
235	P00938	TPIS_HUMAN	Triosephosphate isomerase (TIM)	27.0/6.89	29.0/7.05	67.1
246	Q00688	FKB3_HUMAN	Rapamycin-selective 25 kDa immunophilin (FKBP25)	25.2/10.07	30.2/9.11	17.9
255	P05092	CYPH_HUMAN	Peptidyl-prolyl cis-trans isomerase A (PPIase)	11.2/7.68	16.6/7.53	45.9
256	P05092	CYPH_HUMAN	Peptidyl-prolyl cis-trans isomerase A (PPIase)	18.2/7.83	16.3/7.85	40.0
259	P20071	FKB1_HUMAN	FK506-binding protein 1A; FK506-binding protein 1A	11.9/8.08	10.2/8.35	5.6
261	P23284	CYPB_HUMAN	Peptidyl-prolyl cis-trans isomerase B precursor (PPIase	22.8/10.11	22.8/9.10	43.8
295	Q14376	GALE_HUMAN	UDP-glucose 4-epimerase (Galactowaldenase)	38.7/6.7	37.1/6.65	15.2
305	P30101	PDA3_HUMAN	Protein disulfide isomerase A3 precursor	57.2/6.29	56.6/7.15	23.2
305	P30101	PDA3_HUMAN	Protein disulfide isomerase A3 precursor	57.2/6.29	56.6/7.15	23.2
308	P30405	CYPM_HUMAN	Peptidyl-prolyl cis-trans isomerase	22.7/9.72	22.4/8.82	7.7
311	P05092	CYPH_HUMAN	Peptidyl-prolyl cis-trans isomerase A (PPIase)	11.2/7.68	16.6/7.53	14.6
Select calcium binding						
37	P06396	GELS_HUMAN	Gelsolin precursor, plasma (ADF)	86.1/5.90	88.6/5.9	13.6
72	Q15293	RCN1_HUMAN	Reticulocalbin 1 precursor	38.9/4.71	43.5/4.54	19.9

Table 1 (*Continued*)

Spot no.[a]	Access. no.[b]	Swiss-prot name[c]	Protien description[d]	Theoretical[e] (Mr/p*I*)	Experimental[f] (Mr/p*I*)	Sequence[g] coverage (%)
83	P20073	ANX7_HUMAN	Annexin A7	50.3/6.25	53.7/6.05	7.6
99	P50995	ANXB_HUMAN	Annexin A11 (Annexin XI)	54.7/7.66	56.5/7.36	32.9
129	P07355	ANX2_HUMAN	Annexin II (Lipocortin II)	38.8/7.82	38.5/8.46	45.4
137	P07355	ANX2_HUMAN	Annexin II (Lipocortin II)	38.8/7.82	38.6/7.61	41.3
138	P07355	ANX2_HUMAN	Annexin II (Lipocortin II)	38.8/7.82	36.4/7.00	58.7
139	P07355	ANX2_HUMAN	Annexin II (Lipocortin II)	38.8/7.82	35.7/7.61	58.7
142	P04083	ANX1_HUMAN	Annexin I (Lipocortin I)	38.9/7.02	36.7/6.81	78.3
143	P04083	ANX1_HUMAN	Annexin I (Lipocortin I)	38.9/7.02	36.7/7.02	78.3
150	P20073	ANX7_HUMAN	Annexin A7 (Annexin VII)	50.6/6.55	50.3/6.25	8.9
169	P27797	CRTC_HUMAN	Calreticulin precursor	48.3/4.12	69.0/3.98	20.9
172	P08758	ANX5_HUMAN	Annexin V (Lipocortin V)	36.0/4.76	33.9/4.95	52.8
182	P04632	CANS_HUMAN	Calcium-dependent protease, small subunit	28.5/4.91	29.5/4.95	28.4
184	P12429	ANX3_HUMAN	Annexin III (Lipocortin III)	36.5/5.76	34.1/5.52	56.0
186	P12429	ANX3_HUMAN	Annexin III (Lipocortin III)	36.5/5.76	34.2/5.71	65.6
187	P09525	ANX4_HUMAN	Annexin A4 (Annexin IV)	36.1/5.95	33.5/5.85	68.3
216	P19105	MLRM_HUMAN	Myosin regulatory light chain 2	19.7/4.48	24.2/4.68	18.8
262	P02769	ALBU_BOVIN	Serum albumin	69.3/5.82	69.4/5.72	45.0
263	P02769	ALBU_BOVIN	Serum albumin	69.3/5.82	69.4/5.84	55.6
264	P02769	ALBU_BOVIN	Serum albumin	69.3/5.82	69.4/5.93	36.3
265	P07355	ANX2_HUMAN	Annexin II (Lipocortin II)	38.8/7.82	36.1/7.25	41.3
Select regulatory molecule						
17	P30153	2AAA_HUMAN	Serine/threonine protein phosphatase 2A	66.0/4.81	63.0/5.00	25.1
19	P31150	GDIA_HUMAN	RAB GDP dissociation inhibitor alpha (RAB GDI alpha)	51.2/4.84	58.5/5.05	17.2
81	P50395	GDIB_HUMAN	RAB GDP dissociation inhibitor beta (RAB GDI beta)	51.1/6.15	51.3/6.32	14.6
81	P50395	GDIB_HUMAN	RAB GDP dissociation inhibitor beta (RAB GDI beta)	51.1/6.15	51.3/6.32	33.0
92	Q8WWP7	IMP1_HUMAN	Immunity-associated protein 1	34.4/9.11	55.8/6.80	4.3
103	P49411	EFTU_HUMAN	Elongation factor Tu, mitochondrial precursor (P43)	49.9/7.6	48.2/6.80	11.5
153	P30740	ILEU_HUMAN	Leukocyte elastase inhibitor (LEI)	42.9/6.21	44.6/6.16	43.3
170	P29692	EF1D_HUMAN	Elongation factor 1-delta (EF-1-delta)	31.3/4.97	36.4/4.89	25.6
171	P29692	EF1D_HUMAN	Elongation factor 1-delta (EF-1-delta)	31.3/4.97	36.4/4.97	17.1
179	P29312	143Z_HUMAN	14-3-3 Protein zeta/delta	27.9/4.54	30.1/4.65	100
179	P31946	143B_HUMAN	14-3-3 Protein beta/alpha	28.2/4.58	30.1/4.65	52.8
179	P27348	143T_HUMAN	14-3-3 Protein tau	28.0/4.51	30.1/4.65	31..0
179	Q04917	143F_HUMAN	14-3-3 Protein eta (Protein AS1)	28.4/4.58	30.1/4.65	43.1
182	P11016	GBB2_HUMAN	Guanine nucleotide-binding protein G(I)/G(S)/G(T) beta subunit 2	38.1/5.86	29.5/4.95	20.2
248	P17080	RAN_HUMAN	GTP-binding nuclear protein RAN	24.5/7.11	28.6/7.26	30.1
257	P04080	CYTB_HUMAN	Cystatin B (Liver thiol proteinase inhibitor)	11.2/7.68	10.0/7.23	45.9
309	P32889	ARF1_HUMAN	ADP-ribosylation factor 1	20.6/6.36	23.4/6.69	6.9
Transferase						
21	P07237	PDI_HUMAN	Protein disulfide isomerase precursor (PDI)	57.5/4.59	60.5/5.29	32.7
111	Q16851	UDP2_HUMAN	UTP-glucose-1-phosphote uridylyltransferase 2	57.1/7.69	54.4/7.73	18.1
115	P42765	THIM_HUMAN	3-Ketoacyl-CoA thiolase	42.5/8.24	47.0/8.29	21.2
116	P42765	THIM_HUMAN	3-Ketoacyl-CoA thiolase	42.5/8.24	45.9/8.37	21.2
130	Q9Y617	SERC_HUMAN	Phosphoserine aminotransferase (PSAT)	35.5/6.66	42.0/7.56	38.6
134	P17174	AATC_HUMAN	Aspartate aminotransferase, cytoplasmic	46.5/7.00	43.3/7.12	22.5
151	P04181	OAT_HUMAN	Ornithine aminotransferase	48.9/7.03	47.8/6.24	21
163	Q15274	NADC_HUMAN	Nicotinate-nucleotide pyrophosphorylase	31.3/6.16	35.7/5.97	18.5
290	P21266	GTM3_HUMAN	Glutathione S-transferase Mu 3	27.1/5.28	29.1/5.32	68.4
202	P09211	GTP_HUMAN	Glutathione S-transferase P	23.6/5.37	27.3/5.65	37.6
204	P07741	APT_HUMAN	Adenine phosphoribosyltransferase	19.6/5.88	25.7/5.54	24.6
211	P51580	TPMT_HUMAN	Thiopurine *S*-methyltransferase	28.6/6.18	32.1/6.08	22
227	P00491	PNPH_HUMAN	Purine nucleoside phosphorylase	32.4/6.94	33.5/6.68	69.6
292	P11908	KPR2_HUMAN	Phosphoribosyl pyrophosphate synthetase 2	34.7/6.15	34.7/6.63	36.4
Hydrolase						
4	P27815	CN4A_HUMAN	cAMP-specific 3′,5′-cyclic phosphodiesterase 4A	98.8/4.95	103.1/5.7	6.9
6	P55072	TERA_HUMAN	Transitional endoplasmic reticulum ATPase	90.0/4.99	94.0/5.31	38.7
10	P28331	NUAM_HUMAN	NADH-ubiquinone oxidoreductase 75 kDa subunit	80.6/5.98	80.3/5.51	21.7
35	P09960	LKHA_HUMAN	Leukotriene A-4 hydrolase	69.9/6.12	69.3/6.11	9.5
48	Q16555	DPY2_HUMAN	Dihydropyrimidinase related protein-2	62.8/6.34	64.0/6.41	24.8
49	Q16555	DPY2_HUMAN	Dihydropyrimidinase related protein-2	62.8/6.34	64.2/6.28	40.7
61	P06576	ATPB_HUMAN	ATP synthase beta chain	56.6/5.17	53.5/5.07	44.8

Table 1 (*Continued*)

Spot no.[a]	Access. no.[b]	Swiss-prot name[c]	Protien description[d]	Theoretical[e] (Mr/p*I*)	Experimental[f] (Mr/p*I*)	Sequence[g] coverage (%)
64	P06576	ATPB_HUMAN	ATP synthase beta chain	56.6/5.17	49.4/5.43	50.3
75	Q9Y2T3	GUAD_HUMAN	Guanine deaminase (Guanase)	51.5/5.52	50.1/5.58	37.9
100	P00367	DHE3_HUMAN	Glutamate dehydrogenase 1	61.7/7.82	54.1/7.17	12.2
152	P23526	SAHH_HUMAN	Adenosylhomocysteinase	48.3/6.44	47.3/6.41	12.7
183	Q15181	IPYR_HUMAN	Inorganic pyrophosphatase	33.1/5.68	34.8/5.50	64.7
185	Q15181	IPYR_HUMAN	Inorganic pyrophosphatase	33.1/5.68	34.7/5.64	53.6
272	P10768	ESTD_HUMAN	Esterase D	32.0/7.0	33.3/6.90	15.2
Protease						
4	P27815	CN4A_HUMAN	cAMP-specific 3′,5′-cyclic phosphodiesterase 4A	98.8/4.95	103.1/5.7	6.9
180	P28066	PSA5_HUMAN	Proteasome subunit alpha type 5	26.4/4.74	29.2/4.52	59.0
191	P07339	CATD_HUMAN	Cathepsin D precursor	45.1/6.5	31.6/5.38	23.1
213	P49720	PSB3_HUMAN	Proteasome (prosome, macropain) subunit, beta type, 3	22.9/6.14	27.3/6.28	43.0
214	P49721	PSB2_HUMAN	Proteasome (prosome, macropain) subunit, beta type, 2	22.8/6.52	26.0/6.53	43.0
215	P20618	PSB1_HUMAN	Proteasome subunit beta type 1	26.7/8.19	26.0/6.53	17.4
228	P25786	PSA1_HUMAN	Proteasome subunit alpha type 1	29.8/6.6	33.2/6.60	21.3
238	P25789	PSA4_HUMAN	Proteasome subunit alpha type 4	29.8/7.74	30.6/7.61	16.9
245	O15354	PSA7_HUMAN	Proteasome subunit alpha type 7	28.1/8.37	29.5/8.69	27.8
250	P25787	PSA2_HUMAN	Proteasome subunit alpha type 2	26.0/7.51	27.7/7.07	23.9
252	P20618	PSB1_HUMAN	proteasome subunit, beta type, 1	26.7/8.19	27.2/8.23	24.2
293	P25788	PSA3_HUMAN	Proteasome subunit alpha type 3	28.5/5.07	31.5/5.22	17.3
Kinase						
240	P06732	KCRM_HUMAN	Creatine kinase, M chain	43.1/6.77	30.4/7.98	40.0
274	P22392	NDKB_HUMAN	Nucleoside diphosphate kinase B	17.4/8.52	17.5/8.75	54.6
108	P14618	KPY1_HUMAN	Pyruvate kinase, M1 isozyme	58.4/7.62	59.0/7.65	23.7
110	P14618	KPY1_HUMAN	Pyruvate kinase, M1 isozyme	58.4/7.62	58.4/7.88	62.1
117	P00558	PGK1_HUMAN	Phosphoglycerate kinase 1	45.1/8.11	45.1/8.42	31.8
158	P51570	GAL1_HUMAN	Galactokinase (Galactose kinase)	42.7/6.45	43.0/6.30	43.1
162	O00764	PDXK_HUMAN	Pyridoxine kinase (Pyridoxal kinase)	35.3/6.07	36.7/5.84	26.0
225	P15531	NDKA_HUMAN	Nucleoside diphosphate kinase A	17.3/6.13	23.3/5.94	28.3
241	P54819	KAD2_HUMAN	Adenylate kinase isoenzyme 2	26.7/7.84	30.0/8.06	15.5
247	Q9UIJ7	KAD3_HUMAN	GTP: AMP phosphotransferase mitochondrial	25.6/10.14	28.8/9.11	20.7
292	P11908	KPR2_HUMAN	Phosphoribosyl pyrophosphate synthetase 2	34.7/6.15	34.7/6.63	31.5
Lyase						
33	P48163	MAOX_HUMAN	NADP-dependent malic enzyme (NADP-ME)	64.7/6.04	63.1/5.94	10.1
88	P06733	ENOA_HUMAN	Alpha enolase	47.5/7.38	52.2/6.45	47.5
102	P06733	ENOA_HUMAN	Alpha enolase	47.5/7.38	51.3/6.80	21.4
106	P07954	FUMH_HUMAN	Fumarate hydratase, mitochondrial precursor (Fumarase)	54.8/9.36	48.9/7.46	12.7
118	P04075	ALFA_HUMAN	Fructose-bisphosphate aldolase A	39.9/8.06	40.8/8.36	67
119	P04075	ALFA_HUMAN	Fructose-bisphosphate aldolase A	39.9/8.06	41.2/8.53	47.5
128	P06733	ENOA_HUMAN	Enolase 1; phosphopyruvate hydratase	47.5/7.38	43.0/8.07	67.8
129	O75390	CISY_HUMAN	Citrate synthase, mitochondrial precursor	51.7/8.45	46.4/7.74	37.6
198	Q04760	LGUL_HUMAN	Lactoylglutathione lyase	20.8/5.15	27.3/4.97	44.6
287	Q99798	ACON_HUMAN	Aconitate hydratase	86.2/7.57	85.3/7.36	24.6
288	Q99798	ACON_HUMAN	Aconitate hydratase	86.2/7.57	85.3/7.36	33.1
Synthase and synthetase						
41	P41250	SYG_HUMAN	Glycyl-tRNA synthetase	78.2/6.17	78.2/6.18	29.9
61	P06576	ATPB_HUMAN	ATP synthase beta chain	56.6/5.17	53.5/5.07	44.8
64	P06576	ATPB_HUMAN	ATP synthase beta chain	56.6/5.17	49.4/5.43	50.3
90	P49591	SYS_HUMAN	Seryl-tRNA synthetase	59.3/6.39	59.2/6.46	28.6
112	P22234	PUR6_HUMAN	Multifunctional protein ADE2	47.1/6.94	48.2/7.27	31.5
129	O75390	CISY_HUMAN	Citrate synthase	51.7/8.45	46.4/7.74	35.9
285	P47897	SYQ_HUMAN	Glutaminyl-tRNA synthetase	88.7/7.13	87.6/7.19	47.5
302	P23381	SYW_HUMAN	Tryptophanyl-tRNA synthetase	53.5/6.16	56.6/6.42	32.7
306	P22234	PUR6_HUMAN	Multifunctional protein ADE2	47.8/7.17	47.5/7.08	31.5
Cell adhesion molecule						
42	P35221	CTN1_HUMAN	Alpha-1 catenin	100.8/6.2	98.8/6.16	14.3
43	P18206	VINC_HUMAN	Vinculin	117.3/5.51	110.1/6.21	8.4
44	P18206	VINC_HUMAN	Vinculin	117.3/5.51	110.1/6.26	8.4
220	P09382	LEG1_HUMAN	Galectin-1	14.9/5.23	11.3/4.89	73.9
244	P17931	LEG3_HUMAN	Galectin-3	26.3/9.12	30.3/8.64	28.0

Table 1 (*Continued*)

Spot no.[a]	Access. no.[b]	Swiss-prot name[c]	Protien description[d]	Theoretical[e] (Mr/p*I*)	Experimental[f] (Mr/p*I*)	Sequence[g] coverage (%)
276	Q14126	DSG2_HUMAN	Desmoglein 2 precursor	123.1/5.03	90.0/5.41	19.7
Phosphatase						
155	P08129	PP1A_HUMAN	Serine/threonine protein phosphatase PP1-alpha 1	38.3/6.26	37.5/6.03	22.4
155	P36873	PP1G_HUMAN	Serine/threonine protein phosphatase PP1-gamma	37.7/6.49	37.5/6.03	19.8
155	P37140	PP1B_HUMAN	Serine/threonine protein phosphatase PP1-beta	38.0/6.08	37.5/6.03	23.9
156	P37140	PP1B_HUMAN	Serine/threonine protein phosphatase PP1-beta	38.0/6.08	38.1/6.13	24.5
Signaling molecule						
294	O43399	TD54_HUMAN	Tumor protein D54	22.3/5.11	30.6/5.32	45.1
220	P09382	LEG1_HUMAN	Galectin-1	14.9/5.23	11.3/4.89	73.9
244	P17931	LEG3_HUMAN	Galectin-3	26.3/9.12	30.3/8.64	28
268	Q15691	MAE1_HUMAN	Microtubule-associated protein RP/EB family member 1	30.2/4.86	33.3/5.17	26.9
Ion channel						
96	Q02641	CCB1_HUMAN	Voltage-dependent calcium channel beta-1b subunit	65.7/6.33	70.0/7.19	10.8
189	O00299	CLI1_HUMAN	Chloride intracellular channel protein 1	27.3/4.93	32.2/5.22	57.3
236	P45880	POR2_HUMAN	Voltage-dependent anion-selective channel protein 2	38.7/6.72	33.4/7.47	29.3
242	P21796	POR1_HUMAN	Voltage-dependent anion-selective channel protein 1	30.9/9.04	32.9/8.79	25.1
Ligase						
3	P22314	UBA1_HUMAN	Ubiquitin-activating enzyme E1	117.8/5.49	103.0/5.12	3.8
120	P00966	ASSY_HUMAN	Argininosuccinate synthase	46.7/8.33	46.2/8.06	25.2
200	P27924	UBC1_HUMAN	Ubiquitin-conjugating enzyme E2-25 kDa	22.5/5.21	27.5/5.30	19
Transcription factor						
85	Q9UQ80	P2G4_HUMAN	Proliferation-associated 2G4, 38 kD	43.8/6.13	51.3/6.32	12.5
89	P78381	UGAT_HUMAN	UDP-galactose translocator	41.3/9.98	54.7/6.38	11.8
Extracellular matrix						
43	P18206	VINC_HUMAN	Vinculin	117.3/5.51	110.1/6.21	8.4
44	P18206	VINC_HUMAN	Vinculin	117.3/5.51	110.1/6.26	8.4
Immunity and defense						
13	P11142	HS7C_HUMAN	Heat shock cognate 71 kDa protein	71.1/5.25	70.8/5.46	51.1
296	P11142	HS7C_HUMAN	Heat shock cognate 71 kDa protein	71.2/5.25	72.5/6.41	34.5
300	P11142	HS7C_HUMAN	Heat shock cognate 71 kDa protein	71.2/5.25	72.0/5.39	46.4
Transfer/carrier protein						
111	Q16851	UDP2_HUMAN	UTP-glucose-1-phosphate uridylyltransferase 2	57.1/7.69	54.4/7.73	18.1
226	Q01469	FABE_HUMAN	Fatty acid-binding protein, epidermal	15.5/6.96	13.5/6.35	39.3
Defense/immunity protein						
176	Q07021	MA32_HUMAN	Complement component 1, q subcomponent binding protein	31.3/4.74	33.2/3.98	13.2
Signal transduction						
278	P52888	MEPD_HUMAN	Thimet oligopeptidase	79.6/5.96	84.1/6.04	35.3
Nucleic acid						
243	P09651	ROA1_HUMAN	Heteogeneous nuclear ribonucleoprotein A1	39.0/10.06	33.6/9.13	45.0
312	Q93079	H2BJ_HUMAN	Histone H2B.j (H2B/j)	13.8/11.13	14.7/6.70	23.5
Phosphate						
17	P30153	2AAA_HUMAN	Serine/threonine protein phosphatase 2A	66.0/4.81	63.0/5.00	25.1
Receptor						
132	O96008	OM40_HUMAN	Probable mitochondrial import receptor subunit TOM40	38.2/7.25	41.6/7.43	18.0
Unknown						
4	P54814	SMOO_HUMAN	Smoothelin	99.0/8.65	103.1/5.7	8.6
9	Q14166	Y153_HUMAN	Hypothetical protein KIAA0153	74.5/5.4	86.2/5.42	11.8
14	Q13409	DY12_HUMAN	Dynein intermediate chain2, cytosolic	64.1/4.84	77.7/ 5.29	19.0
53	Q13228	SBP1_HUMAN	Selenium-binding protein 1	52.9/6.56	54.8/6.18	43.9
62	O60268	Y513_HUMAN	Hypothetical protein KIAA0513	46.6/4.98	50.1/5.14	12.4
63	O60664	TI47_HUMAN	Cargo selection protein TIP47	47.2/5.21	50.0/5.27	21.4
154	P37837	TAL1_HUMAN	Transaldolase	37.7/6.8	45.3/6.43	27.9
157	P37837	TAL1_HUMAN	Transaldolase	37.7/6.8	40.5/6.40	15.7
160	094760	DDH1_HUMAN	NG,NG-dimethylarginine dimethylaminohydlase 1	31.5/5.76	36.4/5.68	41.4
165	P50402	EMD_HUMAN	Emerin	29.1/5.25	39.1/4.59	34.6
169	P39149	ALU7_HUMAN	Alu subfamily SQ sequence contamination warning	54.4/11.29	69.0/3.98	23.0

Table 1 (*Continued*)

Spot no.[a]	Access. no.[b]	Swiss-prot name[c]	Protien description[d]	Theoretical[e] (Mr/p*I*)	Experimental[f] (Mr/p*I*)	Sequence[g] coverage (%)
199	P52565	GDIR_HUMAN	Rho GDP-dissociation inhibitor 1 (Rho GDI1)	23.3/4.85	28.0/5.05	48.0
205	P55957	BID_HUMAN	BH3 interacting domain death agonist;	22.0/5.27	25.6/5.40	7.0
206	13129100	ND	Hypothetical protein MGC5627	18.7/4.93	25.7/5.03	31.7
207	13129100	ND	Hypothetical protein MGC5627	18.7/4.93	25.7/5.03	31.7
217	7657176	ND	Transmembrane protein; putative typeII membrane	20.6/4.81	24.5/4.86	32.4
218	17462847	ND	Similar to putative	17.7/4.99	18.8/4.81	11.6
219	P47813	IF1A_HUMAN	Eukaryotic translation initiation factor 1A(EIF-1A)	16.4/4.92	12.7/4.90	54.5
221	47592121	ND	Beta-tubulin cofactor A	12.8/5.25	12.7/5.33	43.0
222	7705477	ND	Hypothetical protein	14.2/5.21	12.4/5.52	75.0
223	Q14259	ERH_HUMAN	Enhancer of rudimentary homolog	12.5/5.77	9.81/5.54	5.8
269	Q96C19	SWS_HUMAN	Swiprosin 1	26.8/4.97	33.0/5.04	29.6
297	Q12931	TRAL_HUMAN	Heat shock protein 75 kDa, mitochondrial precursor	80.3/8.12	75.4/6.52	25.7
310	D15509	AR20_HUMAN	APR2/3 complex 20 kDa subunit (P20-ARC)	19.8/8.65	20.0/8.65	21.4
45	18042949	ND	Unknown MGC: 17003	96.0/6.13	97.8/6.44	28.0
55	P31934	ROH1_HUMAN	Heterogeneous nuclear ribonucleoprotein H (hnRNP H)	49.5/6.26	53.3/6.08	48.6
60	Q12849	GRF1_HUMAN	G-rich sequenc factor-1 (GRSF-1)	48.5/5.59	55.2/5.25	12.1
64	P52597	ROF_HUMAN	Heterogeneous nuclear ribonucleoprotein F (hnRNP F)	46.0/5.39	49.4/5.43	26.3
79	P31943	ROH1_HUMAN	Heterogeneous nuclear ribonucleoprotein H (hnRNP H)	49.5/6.26	55.2/5.25	21.2
84	P35998	PRS7_HUMAN	26S protease regulatory subunit 7	49.0/5.73	51.3/5.97	29.6
113	P25705	ATPA_HUMAN	ATP synthase alpha chain, mitochondrial precursor	59.9/9.93	53.6/8.03	14.3
131	Q92524	PRSX_HUMAN	26S protease regulatory subunit S10B	44.4/7.49	44.7/7.39	25.4
146	P06748	NPM_HUMAN	Nucleophosmin (NPM)	32.7/4.47	38.9/5.42	29.3
147	Q03154	ACY1_HUMAN	Aminoacylase-1 (*N*-acyl-L-amino-acid amidohydrolase)	46.1/6.13	45.8/6.04	14.5
166	P13489	RINI_HUMAN	Placental ribonuclease inhibitor	51.8/4.54	51.2/4.95	19.9
167	Q09028	RB48_HUMAN	Chromatin assembly factor 1 P48 subunit	47.9/4.59	53.9/4.54	13.9
178	P31974	143S_HUMAN	14-3-3 protein sigma (Stratifin)	27.9/4.5	31.1/4.41	73.8
192	P35232	PHB_HUMAN	Prohibitin	29.9/5.55	31.2/5.60	59.9
196	P30040	ER29_HUMAN	Endoplasmic reticulum protein Erp29 precursor	29.1/7.46	29.9/5.90	33.7
201	Q9Y5S9	RBM8_HUMAN	RNA-binding protein 8	19.9/5.52	26.1/5.26	23.6
208	P30040	ER29_HUMAN	Endoplasmic reticulum protein Erp29 precursor	29.1/7.46	30.1/6.29	26.4
251	P30086	PEBP_HUMAN	Phosphatidylethanolamine-binding protein	20.9/7.43	26.0/7.74	7.9
258	P08206	S110_HUMAN	Calpactin I light chain (P11)	11.2/7.44	8.6/7.63	59.4
269	Q99426	TBCB_HUMAN	Tubulin-specific chaperone B (Tubulin folding cofactor)	27.6/4.89	33.0/5.04	29.9
276	P13798	ACPH_HUMAN	Acylamino-acid-releasing enzyme	82.3/5.25	90.0/5.41	20.1
289	P52272	ROM_HUMAN	Heterogeneous nuclear ribonucleoprotein M (hnRNP M)	77.6/9.35	75.8/8.65	33.3
290	P52272	ROM_HUMAN	Heterogeneous nuclear ribonucleoprotein M (hnRNP M)	77.6/9.35	74.7/8.78	33.3
291	P52272	ROM_HUMAN	Heterogeneous nuclear ribonucleoprotein M (hnRNP M)	77.6/9.35	74.8/8.88	33.3
303	P43686	PRS6_HUMAN	26S protease regulatory subunit 6B	47.5/4.94	51.4/5.20	31.6
303	P17980	PRSA_HUMAN	26S protease regulatory subunit 6A	49.4/4.92	51.4/5.20	60.8
197	P13693	TCTP_HUMAN	Translationally controlled tummo protein (TCTP)(P23)	19.7/4.67	27.1/4.77	39.0
186	095994	ND	Anterior gradient 2 homolog	20.0/9.03	16.5/8.99	9.9

[a]Spot numbers (spot nos.) correspond to those in Fig. 2. [b]Accession number, [c]Swiss-prot name, [d]protein description, [e]theoretical molecular weight and isoelectric point (theoretical Mr/p*I*), [f]observed molecular weight and isoelectric point (experimental Mr/p*I*) were obtained based on the location of the spots on the 2D gel. [g]The protein amino acid sequence coverage by the matching peptides (Sequence coverage (%)).

were compared (Fig. 3A and B, respectively). The overall differences were estimated by scatter plot analysis (Fig. 3C), showing that the intensity of 28.0% of the protein spots changed more than four-fold after treatment with phosphatase. Examples of the protein spots whose intensity was affected more than four-fold by phosphatase treatment are shown in Fig. 3D. As phosphorylation of a protein changes its p*I* and consequently its position in 2D gels, phosphatase treatment results in alterations of spot intensity. It is possible that some phosphorylated proteins might be resistant to phosphatase treatment and the number of phosphorylated proteins might be more than expected by this experiment. We considered that spots with decreased intensity after phosphatase treatment were probably phosphorylated in their original location on the 2D gel. On the other hand, we considered that spots with increased intensity after phosphatase treatment represent migration of the previously phosphorylated isoform to the location of the unphosphorylated isoform. The protein spots whose intensity was affected by phosphatase treatment are listed in Table 4. The proteins corresponding to these spots were identified by matching 2D iamge of experimental samples to the reference gel image (Fig. 2).

3.4. Application of the 2D database of Cy5-labeled proteins to colon cancer tissue

We compared the 2D profile of the colon cancer cell line DLD-1 with that of colon cancer cells obtained from surgical

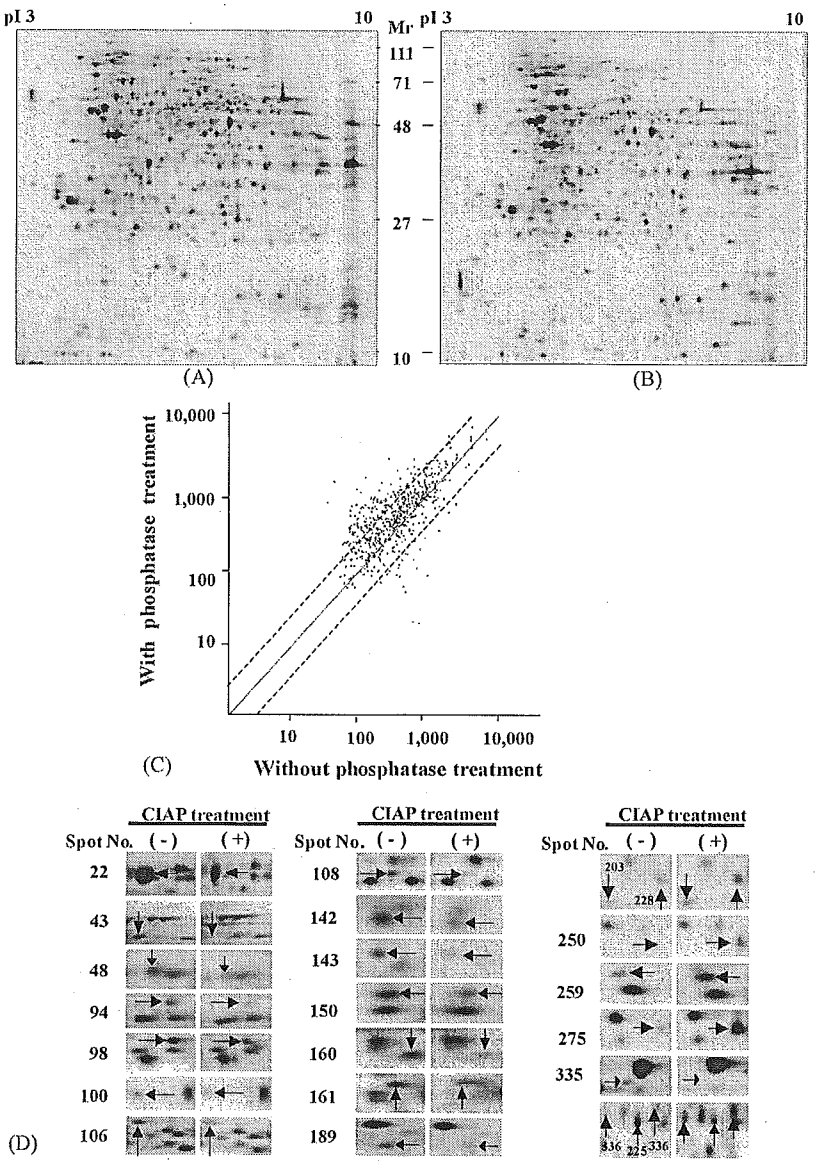

Fig. 3. Effects of phosphatase treatment on the intensity of the protein spots of DLD-1 cells. A protein lysate of DLD-1 cells was incubated with (A) and without (B) alkaline phosphatase, and the proteins were labeled with Cy3 and separated by 2D-PAGE. (C) The overall changes as a result of phosphatase exposure were estimated by scatter plot analysis with (x-axis) and without (y-axis) phosphatase exposure. The broken lines represent four-fold differences from the ($y = x$ axis). (D) The 2D images of the proteins with (+) and without (−) phosphatase are summarized. The arrows point to the protein spots whose intensity was changed more than four-fold by phosphatase treatment. The numbers correspond to those in Table 1, and the results are summarized in Table 4.

specimens by laser microdissection. Tissue sections were briefly stained with hematoxylin (Fig. 4A), and the cancer cells were isolated by laser microdissection (Fig. 4B). The overall 2D protein profiles of the DLD-1 cells (Fig. 4C) and the colon cancer cells (Fig. 4D) were compared by scatter plot analysis (Fig. 4E). To evaluate the similarity of these two 2D images, we performed the scatter plot analysis. Scatter plot analysis revealed that the intensity of 93% of the protein spots from the colon cancer cells was within four-fold of the intensity of the corresponding spots from DLD-1 cells. As most of the protein spots from the colon cancer cells were present on 2D image of DLD-1 cells, it could be possible to identify the proteins corresponding to the spots of colon cancer cells by matching the 2D image with the reference image in our database.

4. Discussion

We constructed a 2D database of fluorescence-labeled proteins of colon cancer cells. This is the first 2D database of fluorescence-labeled proteins of a higher eukaryotic proteome. Moreover, this is one of the largest databases of colon cancer cells, comprising 258 different gene products. To detect protein spots, we used currently developed fluorescent dye. The different detection methods may result in different

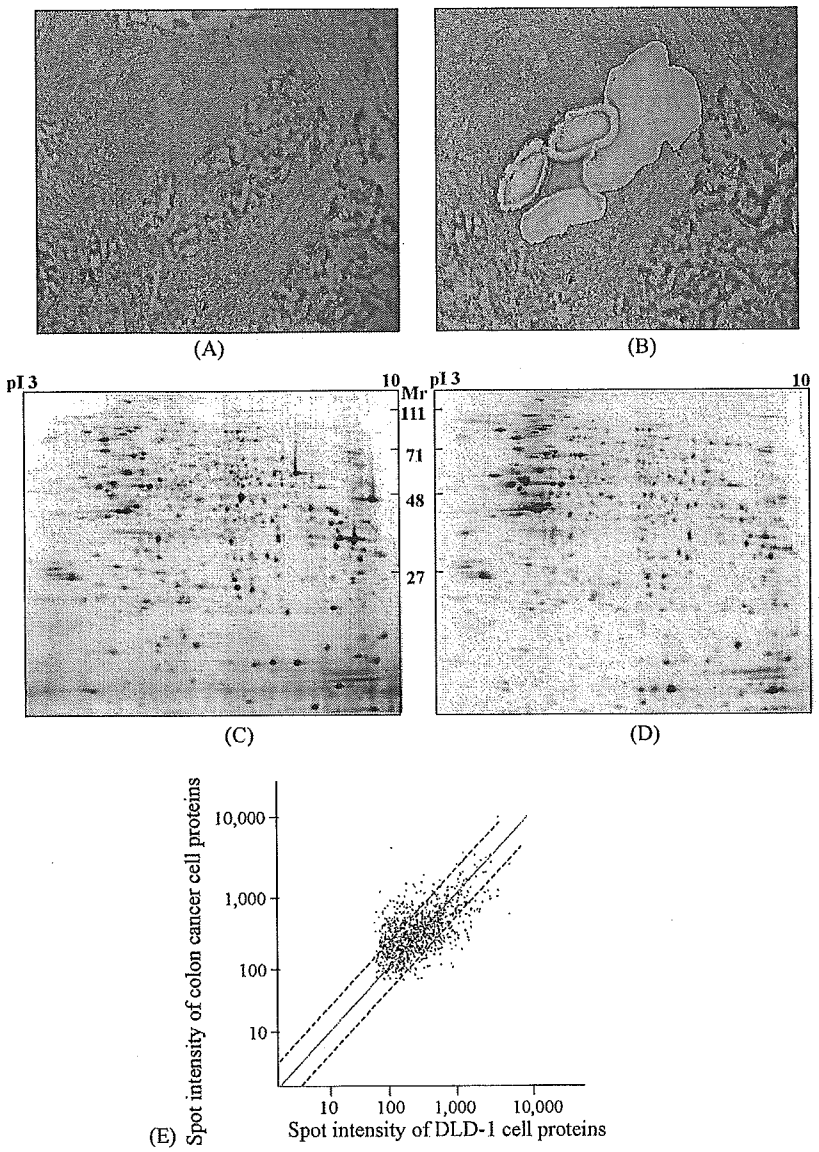

Fig. 4. Frozen colon cancer tissues were sectioned and stained with hematoxylin (A), and the tumor cells were recovered by laser microdissection (B). Overall comparison of the 2D profile of colon cancer cells (C) with that of DLD-1 cells (D). The coefficient was determined by scatter plot analysis. The broken lines represent four-fold differences from the ($y = x$ axis). The correlation coefficient (r) is 0.5.

2D images, due to the different specificities of the detection method. For example, the Cy5 fluorescent dye binds lysine residues of proteins [5], whereas SYPRO Ruby has strong affinity for lysine, arginine, and histidine residues and weak affinity for tyrosine and tryptophan residues [17]. Currently, several 2D databases for colon cancer cells [19–22] and a comparative proteomic study of colorectal carcinoma using 2D-PAGE [23–26] have been reported. We found that it was difficult to match the 2D images from these studies with the reference 2D image in our database, perhaps because of differences in protocols for protein extraction, electrophoresis and spot detection. However, because we used commercially available IPG gels and the results are highly reproducible, our database will be useful when an identical protocol is used to generate the 2D gel.

Functional classification of the identified proteins revealed that 42.5% of them could be categorized into the oxidoreductase, cytoskeletal protein, nucleic acid binding protein, chaperone, and isomerase families. Therefore, 2D-DIGE will be useful for studies focusing on these protein families, whereas proteins with lower expression levels will require more sensitive detection methods. We found that 52 proteins were represented by multiple spots. The total number of observed spots is approximately 1500 with our protocol. Consequently, assuming that the rate of spot redundancy, 258 proteins/314 spots, is similar for the rest of the protein spots, the total number of proteins in our database can be estimated as 1232. These results suggest that modified procedures will be required to enrich this small observed fraction of the proteome, because the total number of gene products

Table 2
Percentages of protein spots according to protein category

Protein category	Spots	Percentage (%)
Oxidoreductase	39	10.5
Cytoskeletal protein	35	9.4
Nucleic acid binding	33	8.9
Chaperone	26	7.0
Isomerase	25	6.7
Select calcium binding	22	5.9
Miscellaneous function	18	4.9
Select regulatory molecule	14	3.8
Transferase	14	3.8
Hydrolase	14	3.8
Protease	12	3.2
Kinase	11	3.0
Lyase	11	3.0
Synthase and synthetase	9	2.4
Cell adhesion molecule	6	1.6
Phosphatase	4	1.1
Signaling molecule	4	1.1
Ion channel	4	1.1
Ligase	3	0.8
Immunity and defense	3	0.8
Transcription factor	2	0.5
Extracellular matrix	2	0.5
Transfer/carrier protein	2	0.5
Nucleic acid	2	0.5
Defense/immunity protein	1	0.3
Signal transduction	1	0.3
Phosphate	1	0.3
Receptor	1	0.3
Unknown	52	14.0
Total	371	100.0

in any given cell is estimated to be around 10,000 and post-translational modifications of gene products should increase the total number of protein isoforms [27]. Fractionation and concentration of particular fractions of the proteome prior to electrophoresis [28], labeling of proteins with radioactivity [29], large format 2D gels [30] and combination of narrow-p*I* range IPG gels [31] can be used to increase the number of observable spots. To allow observation of a greater fraction of the proteome, there have also been intense efforts to develop mass spectrometry-based proteomic approaches complementary to 2D-PAGE. However, none of these technologies appears adequate to uncover the entire proteome, suggesting that a database for each approach will be required to understand its limitations, to avoid redundancy between the multiple methods and to integrate whole data.

Table 3
Proteins matching the same gene in Table 1

Name of protein	No. of isoforms detected
Heat shock cognate 71 kDa protein	5
Annexin II	5
Electron transfer flavoprotein alpha-subunit	4
Protein disulfide isomerase A3 precursor	4
Actin, cytoplasmic 1 (beta-actin)	3
Glyceraldehyde 3-phosphate dehydrogenase	3
Peptidyl-prolyl cis-trans isomerase A (PPIase)	3
Alpha enolase	3
Endoplasmin precursor (GRP94)	3
Heat shock protein HSP 90-alpha (HSP 86)	3
Isocit rate dehydrogenase (NADP) cytoplasmic	3
Eukaryotic translation initiation factor 3 subunit 2	3
Heterogeneous nuclear ribonucleoprotein M (hnRNP M)	3
Keratin, type I cytoskeletal 19 (CK19)	3
Serum albumin precursor	3
Ubiquinol-cytochrome C reductase complex core Protein I	3
Products of 36 different genes (2 spots for the same protein)	72

Summary of the spots identified that originated from the same genes. Of the 314 protein spots identified by mass spectrometry, 52 proteins appeared more than once. The names of the proteins are the same in Table 1.

Table 4
Phosphorylated proteins in Table 1

Spot no.	Protein name
22	60 kda heat shock protein
42	Alpha-1 catenin
95	Elongation factor 2 (EF2)
47	T-complex protein 1, alpha subunit
90	Seryl-trans synthetase
93	Stress induced-phosphoprotein 1
101	Glucose-6-phosphate 1-dehydrogenase (G6PD)
150	Annexin A7
160	Spermidine synthase
103	Elongation factor Tu, mitochondrial precurcer
306	Multifunctional protein ADE2
134	Aspartate aminotransferase, cytoplasmic
133	40 kDa peptidyl-proly cis-trans isomerase
141	60S acidic ribosomal protein P0
177	14-3-3 protein epsilon
229	Eukaryotic translation nitlation factor 4H
272	Esterase D
230	Electron transfer flavoprotein alpha subunit
307	Electron transfer flavoprotein alpha subunit
234	Phosphoglycerate mutase
250	Cofilin

The protein spots whose intensity changed more than four-fold were categorized as phosphorylated proteins. Spot number (spot no.) and protein names are the same as in Table 1.

To evaluate the phosphorylation status of our proteins, we examined the effects of phosphatase treatment on protein migration in the 2D gel. We found that the intensity of 28.0% of the spots was changed more than four-fold by phosphatase treatment. These results appear to differ from those of a recent study in which exposure of protein samples to phosphatase showed that 4.8% of the detectable proteins were phosphorylated [32]. We did not investigate whether this discrepancy was attributable to different conditions of phosphatase exposure or to differences between the proteome observed by fluorescent dye labeling and that observed by conventional colorimetric staining. It is also possible that the phosphorylation status of the proteome varies between different cells, since the previous study used cultured rat skin fibroblasts. Although more than 10% of proteins in a typical mammalian cell are thought to be phosphorylated, the exact number of phosphorylated proteins has not been determined. As the 2D-PAGE with phosphatase treated samples is conventional method to obtain the overview of phosphor-proteome, 2D-PAGE will be a useful tool for an initial step

for phospho-proteomics. Multiplex imaging using different dyes is a powerful tool to compare the samples before and after the treatment, as it can detect the small alterations before and after the treatment without gel-to-gel variations. On the other hand, we need to bare in mind that it is possible that the proteins with exactly same p*I* and MW to some protein might appear by phosphatase treatment. Therefore, other approaches, such as Western blotting with antibodies against phosphorylated proteins and metabolic labeling of the cells with isotopes [33], will be helpful to compliment to the experiment of phosphatase treatment for the phospho-proteome. Mass spectrometry-based approaches will be also effective in studying the phospho-proteome. Shu et al. used liquid chromatography–tandem mass spectrometry to identify 107 phosphorylated proteins in murine B lymphocytes [34]. Integration of the results of gel-based and mass spectrometry-based proteomic approaches will be required to understand the complex phospho-proteome. In the present study, we examined only the phosphorylation status of protein spots. However, the presence of redundant protein spots that were unaffected by phosphatase treatment suggests that post-translational modifications other than phosphorylation such as glycosylation may also contribute to redundancy.

A 2D database is a powerful tool for rapid identification of the proteins corresponding to observed protein spots. The protein corresponding to a spot of interest can be identified by matching the 2D image of the experimental sample with the reference image in the database. We demonstrated that the 2D image of DLD-1 cells was similar to that of colon cancer cells. Therefore, our 2D database will be useful to identify the majority of proteins of colon cancer cells in vivo and can be used for proteomic studies of colon cancer tissues. In many cases, because only a limited amount of clinical material is available, it may be difficult to obtain enough amount of sample such as 500 μg protein for protein identification, especially as a laser-microdissection is employed to obtain the tumor cells. In that case, we can run 2D-PAGE with small amount of protein such as 50 μg to create the protein expression profiles for analytical purpose and, to identify the protein corresponding to the interesting spots, we can match the image of analytical gels to that of the reference image in the database for protein identification. The similarity of 2D images of in vitro and in vivo cells will facilitate the functional study of proteins. When interesting protein spots are found in in vivo cells, we may be able to study what stimuli alter the expression level and posttranslational modifications of the protein spots using in vitro cells. Comparison between the in vitro and in vivo images of 2D-PAGE will allow such study for many proteins simultaneously without specific antibodies.

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プロテオミクスによる癌の悪性形質を裏付けるタンパク質群の同定

オーダーメイド医療のための腫瘍マーカーの開発へ向けた試み

近藤　格

プロテオミクスの分野では技術開発と並行して癌の網羅的タンパク質発現解析が盛んに行われている．臨床応用に最も近い研究例としてはオーダーメイド医療のための腫瘍マーカーの開発があげられる．癌の悪性形質を裏付けるタンパク質群をプロテオミクスの技術で同定し，より正確な診断を行い最適の治療法を選択するための腫瘍マーカーとして使用しようとする試みである．すでに肺癌や脳腫瘍に関しては癌患者の予後を予測できるタンパク質群がプロテオミクスの技術で報告されており，実用化へ向けた発展が期待される．

キーワード●　網羅的タンパク質発現解析，二次元電気泳動，予後，抗癌剤耐性

はじめに

プロテオミクスはinfancyな状態である，とよく語られる．タンパク質の全体像をとらえ，全体像をとらえることでしか得られない知見をもって生命現象を解明する，というプロテオミクス本来のアイデアからすれば，学問としてのプロテオミクスは確かにまだまだ完成度は低い．実際，プロテオミクスの分野で最もホットなトピックスは方法論に関するものであり，確立されて開発がプラトーに達した方法論は未だ存在しない．古典的といわれる二次元電気泳動ですら従来から指摘されていた弱点が克服されるブレークスルーが新しい蛍光試薬によってもたらされ，応用範囲が大きく広がった[1]．一方，派手な宣伝文句とともに登場した技術であっても実際に生物学的なデータを出すにあたって克服するべき問題点が明らかになっている技術もある．技術改良の傍らでは網羅的タンパク質発現解析が進みつつあり，その結果のフィードバックからそれぞれの技術の特性や限界について専門家の間では共通の認識が得られつつある．このような技術開発の時代はこれからもしばらく続くだろう．高額な機械を導入しさえすれば飛躍的に研究が進むとする非現実的な楽観論や，無数にあるタンパク質を包括的に理解することは到底できないとする極端な悲観論は影をひそめ，プロテオミクスの困難さが具体的なイメージを伴って理解されはじめているということが，プロテオミクスのここ数年の最大の進歩である．

癌研究における網羅的タンパク質発現解析は30年前に二次元電気泳動が発表されたころから行われている．ここ数年は最新のプロテオミクスの手法（ゲノムデータベースと質量分析の組合わせによる微量タンパク質の同定技術），いわゆるIT関連技術（大量のデータをネットワーク上で高速に扱う技術とデータマイニングの手法），そしてリファインされた古典的タンパク質分離技術（液体クロマトグラフィー，ゲル電気泳動など）などが組合わされ，従来の発現解析の限界を凌駕する応用が試みられている．癌研究におけるタンパク質発現解析のテーマも，従来は正常組織と癌組織を比較するという大雑把なものだったが，最近では特定の癌の形質に焦点を絞り，癌の複雑な悪性形質（不

Cancer proteomics for personalized medicine

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良な予後，抗癌剤への耐性など）を裏付けるタンパク質群を同定しようとする試みが盛んになっている．その目的は癌のメカニズムの解明に加え，癌の個性をより正確に診断し治療方針の決定に役立つ腫瘍マーカーを開発したり，創薬のターゲットを見つけたりするところにある．本稿では特に臨床応用への指向性が強く著者が興味をもっている，オーダーメイド医療のための腫瘍マーカー開発を紹介する．

1 オーダーメイド医療のための腫瘍マーカー

個人個人に合わせた最適な医療（オーダーメイド医療）を行うためには，癌の個性を正確に診断することが最も重要である．既存の臨床病理学的な診断では同一グループに分類される癌細胞あるいは癌患者が，治療に対して異なる反応性を示すことが一般に知られており，治療方法の決定のためにより正確な診断を可能にする腫瘍マーカーの開発が期待されている．例えば乳癌ではリンパ節の転移を正確に診断できれば，的確な手術法を選択したり早期に治療を開始することが可能である．また肺癌では抗癌剤としてのEGF受容体阻害剤（ゲフェチニブ，商品名イレッサ）が大変よく効く症例がある一方で副作用が強く現れる症例もあり，両群を治療開始前に鑑別できれば治療成績は大きく向上すると考えられている．乳癌患者の予後や肺癌患者のゲフェチニブへの感受性は単一の遺伝子の異常からも予測がつくとする考え方があり，それぞれについてCyclin Eの過剰発現[2]やEGF受容体の異常[3]が報告されている．

しかしながら，これらの異常だけでは予測できない予後の悪い症例や副作用も数多く存在する．予後や抗癌剤への耐性は単一の分子で説明される事象ではなく，おそらく複数の分子が関与しているのだろう．また，遺伝的な背景は患者ごとに異なっているため，予後や抗癌剤の奏効性などを決定するメカニズムそのものにバリエーションがあるかもしれない．そのような観点から，多くの症例をカバーする正確な予測のために遺伝子発現の異常を網羅的に調べ，複数の分子の異常を同時に調べることで癌を多角的に調べようとする試みがある．mRNAの網羅的な発現解析を通して，乳癌患者の予後の予測[4]，肺癌患者のゲフェチニブへの感受性の予測[5]のための腫瘍マーカーの開発が行われている．診断を精緻に行い癌の個性を判別することが治療上大変役立つ事例はほかの悪性腫瘍でも認められており，それぞれについて同様にmRNAの網羅的発現解析が試みられている．これからもmRNAの発現で癌の個性診断を行う研究は増えていくだろうし，その成果を商品化する例も増えるだろう．そのような現状で，プロテオミクスの技術を用いてオーダーメイド医療のための腫瘍マーカーの開発を行うメリットはどこにあるのだろうか？

2 腫瘍マーカー開発におけるプロテオミクスの有用性

網羅的発現解析の成果の一端として，mRNAとタンパク質の発現レベルには相関が乏しいことが知られるようになった．例えば，肺癌において二次元電気泳動とGeneChipを用いてタンパク質とmRNAの発現解析の結果を比較した例では，タンパク質とmRNAの発現レベルが相関していたのは調べた165個のタンパク質のうちの2割ほどだけであった[6]．タンパク質発現解析ツールとしては二次元電気泳動法，抗体アレイ，ICAT（isotope coded affinity tag），質量分析，mRNA発現解析ツールとしてはGeneChipを用いた同じような報告は数多くあり[7)~10)]，いずれもmRNAとタンパク質の発現レベルの相関が著しく乏しいという結果に終わっている．

細胞の形質をコントロールするのはおそらく多くの場合はmRNAではなくタンパク質である．発現解析をmRNAのレベルで行うとき，mRNAとタンパク質の発現レベルにある程度の相関があることが期待されてきたのだが，その期待は裏切られつつある．しかしこれらの事実はmRNAレベルの発現解析が役に立たないことを示唆しているわけでは決してない．DNAマイクロアレイ技術（またはGeneChip）は完成度も高く，網羅性，定量性に優れた技術であり，mRNAとタンパク質の発現に相関がある遺伝子に関しては完成度の高い強力なツールである．しかし，どんなにア

レイする遺伝子を増やしてもタンパク質発現という観点からはすべての遺伝子がカバーされているわけではなく，カバーされていない遺伝子を補完するタンパク質レベルの発現解析も必要だということである．

プロテオミクスの技術を用いれば腫瘍マーカーの開発はmRNAの発現解析以上に効果的に行うことができるだろうと考えている．なぜならば，プロテオミクスでは細胞の機能をコントロールするタンパク質の量を直接調べることができるからである．また，翻訳後修飾（リン酸化，糖鎖修飾など），体液（血清，脳脊髄液など）は癌で異常を来していることが知られており，いずれも癌の個性診断に役立つはずだが，これらはタンパク質を調べなくてはわからない異常だからである．

問題は現在のプロテオミクスの技術レベルにある．プロテオミクスの分野ではDNAマイクロアレイ（またはGeneChip）に相当する，①網羅的で，②定量的であり，③再現性もよく，④多検体を並列に解析でき，⑤統合できるフォーマットで多検体からデータを出せる，という技術はまだ存在しない．プロテオミクスでは，網羅性の高い技術は定量性に乏しく，逆に定量性が高い技術は網羅性に欠ける傾向がある．それぞれに弱点を克服する努力は行われており，新しいアイデア，試薬，機械の導入によってここ数年かなり進歩したが，まだずいぶん開発の余地があるようだ．だからといって現段階でプロテオミクスが癌の研究の役に立たないというわけではない．最先端から一歩下がった市販のプロテオーム解析技術を用いた研究結果からでも，プロテオーム解析を用いた腫瘍マーカー開発の明るい将来性を示唆するデータは出つつある．

3 プロテオーム解析により同定された腫瘍マーカー候補

例えば，肺癌患者の予後と強く相関するタンパク質群が二次元電気泳動や質量分析で同定されている．Hanashのグループは二次元電気泳動法を使い肺腺癌90症例の切除標本を用いてプロテオーム解析を行い，早期の肺腺癌患者の術後の生存期間を予測しうるタンパク質群を同定している[11]．プロテオーム解析に用いられたのは682スポットにすぎないが，結果的には予後予測のための腫瘍マーカーとして臨床応用が可能なタンパク質が見つかっている．予後の悪い症例では解糖系酵素に属する4つのタンパク質の発現が高かったが，そのうちPGK1（phosphoglycerate kinase 1）は血中レベルも予後と相関していたことから，非侵襲的な診断法の開発につながる可能性が高い．その他，予後の悪い症例では，多くの悪性腫瘍で発現減少が報告されているミトコンドリアSODや逆に亢進している例の多いFGF 4（fibroblast growth factor 4），抗凝固作用のあるAnnexin Ⅷの発現が高くなっていた．また，抗アポトーシス作用があるとされるGRP78（glucose regulated protein 78），好中球と反応するGRK4（G protein-coupled-receptor kinase 4），癌遺伝子産物N-mycなどの発現が高い患者の予後がよいという結果になっている．プロテオミクスの最終目標はこれらのタンパク質の異常を包括的に理解し，予後を左右するタンパク質のネットワークを解明することだろう．

しかしそのような大目標達成の前にこれらのタンパク質を腫瘍マーカーとして使用することによって治療成績の向上が見込まれるし，これらを分子標的とした治療法の開発によって予後は大きく改善される可能性がある．柳沢ら（現名古屋大学）も同様に肺の非小細胞癌79症例を対象にMALDI TOF MSを用いたタンパク質の発現解析を報告している．1,600本以上のイオンピークの定量的シグナルを対象に解析を行い，肺癌患者のきわめて悪い予後を予測しうる15本のイオンピークを同定している[12]．また，岩立ら（千葉大学）も二次元電気泳動を用いて75症例の脳腫瘍を調べ，257個のタンパク質スポットから悪性度に相関する37個のスポットを同定している[13]．DNAマイクロアレイ（またはGeneChip）と比べるまでもなく網羅性は低いのだが，定量的にプロテオーム解析を行うことでオーダーメイド医療のための腫瘍マーカー候補が発見されつつある点は注目に値する．別の言い方をすると，今すでにある技術を改良して定量性を保ったまま網羅性を向上させさえすれば，癌のプロテオーム研究は臨床応用，特にオーダーメイド医療のための腫瘍マーカーの開発に向けて大きく進歩できるだろう．

二次元電気泳動に関しては既存の技術の応用で観察できるタンパク質の数を10倍増やすことは容易であ

り，実際にKloseらは10,000個近い数のスポットを解析に用いたプロテオーム解析を数多く報告している[14]．腫瘍マーカーの開発においてプロテオミクス側からのアプローチはトランスクリプトーム側からより本当に有効なのかが今後の研究によって明らかにされていくだろう．また，「オーダーメイド医療のための腫瘍マーカー開発」，という早期に実現可能かつ社会的にインパクトの高い研究テーマが提示されたことで，この方面の技術開発がいっそう進むことを期待している．具体的には，定量性を最も重視しつつ網羅性とスループット性を高めたタンパク質発現解析技術の開発である．

国立がんセンターでも肝癌や肺癌患者の術後再発の予測，肺癌や骨肉腫の抗癌剤への感受性の予測，食道癌や肺癌の微小なリンパ節転移の検出，骨軟部腫瘍の鑑別診断などをめざし，実際の手術検体を多数用いて腫瘍マーカーの開発を行っている．次にそのために使われている定量的プロテオーム解析技術について述べる．

4 腫瘍マーカー開発ツールとしての二次元電気泳動の可能性

二次元電気泳動法はプロテオーム解析の手段として最も普及している技術である．分離されたタンパク質の量を定量的に測定できるというメリットがある．さらに分子量と等電点という，すべてのタンパク質が普遍的かつ固有にもつ物理特性をもとに分離を行うことで，二次元電気泳動法はほかのプロテオーム解析系にはない高い分離能を示している．現在までにさまざまなバックグランドや目的をもつ研究者によって二次元電気泳動法は使用され，あらゆる角度からその特性や限界が検証されてきた．二次元電気泳動で解析が困難な膜タンパク質や微量タンパク質のために，そしてさらに定量性，分離能，再現性を高めるためにさまざまな工夫が行われてきた．工夫の結果は主にElectrophoresis誌に論文として発表されており，新しいアプリケーションを考案する際に大変貴重なデータベースとなっている．もったいないことに単に技術開発として報告されただけで実際に生物学のテーマに応用されていない例が多い．それはスポットに対応するタンパク質が従来のアミノ酸シークエンサーでは効率よく同定することができなかったためなのだが，最近では質量分析によってすべてのスポットの同定が可能になっている．そのため，過去に報告されたアプリケーションはまさにアイデアの宝庫である．一方で昔からある技術の典型例として二次元電気泳動は新しい技術が登場する都度ベンチマークとして使用され，ときとして弱点を誇張する表現とともに取り上げられてきた．限界のない技術はないのだが，本邦では二次元電気泳動の限界は誤って低く理解されているように思われる．

国立がんセンターではオーダーメイド医療のための腫瘍マーカーの開発が二次元電気泳動法を用いて行われている．腫瘍マーカー開発のためにDNAマイクロアレイの前述の5つの性能を目標としてこの数年間工夫を加え続けた結果，二次元電気泳動法の再現性，定量性，スループット性などは飛躍的に向上した[15]．鍵となるのは泳動前にタンパク質を蛍光色素で標識する技術で，すべてのスポットに内部標準をもたせることがポイントである．また，市販の泳動装置（24 cm × 20 cmゲル）でも通常は少なくとも1,500スポットがかなり再現性よく描出されるが，網羅性を向上させるために40 cm × 30 cmという巨大ゲルをハイスループット型の自作電気泳動装置で泳動するようになった．試算では1枚のゲルでSWISS-PROTデータベースの半分以上を定量性を保ってカバーできると考えている．同定感度が上がった結果，最近ではオーファンレセプター，Grb2（growth factor binding protein 2），DNA修復酵素，転写因子または腫瘍抑制遺伝子産物に結合するタンパク質など，微量すぎて従来は二次元電気泳動で観察できないと考えられていたタンパク質がスポットとして次々と同定されている．網羅性を高める工夫はさらに進行中であるが，その傍らでは肝癌の早期再発を予測できるタンパク質群をすでに同定しており，実用化をめざした研究を開始している．

おわりに

この4年間のプロテオーム解析技術の進歩は目覚ましく，市販されている技術を工夫することで医学生物学の研究テーマにあった新しい方法をつくることがで

きる段階にわれわれは到達している．癌研究の分野では，腫瘍マーカーの開発だけでなくメカニズムの解明，予防，早期発見，治療法の開発など，臨床応用をにらんだ実にさまざまな研究テーマがある．癌の悪性形質を裏付けるタンパク質群を網羅的に同定し，最終的にはそれらのネットワークを解明することで，既存のタンパク質研究にはなかったプロテオミクスならではの癌研究を行いたいと考えている．

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5章　バイオインフォマティックス

コラム

バイオインフォマティックスを用いた疾患Omicsによる疾患マーカーの開発

近藤　　格

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