Table 2. List of proteins identified by mass spectrometry

Spot no.[a]	Prot_acc[b]	Identified protein[b]	Prot_pi[c]	Prot_mass[c]	Prot_score[d]
500	ANXA6_HUMAN	Annexin A6	5.42	76168	628
1725	CLUS_HUMAN	Clusterin precursor	5.89	53031	109
563	GRP75_HUMAN	Stress-70 protein	5.87	73920	930
1688	CLC11_HUMAN	C-type lectin domain family 11 member A	5.06	36015	198
57	VIME_HUMAN	Vimentin	5.06	53676	373
11	VIME_HUMAN	Vimentin	5.06	53676	775
2003	HBB_HUMAN	Hemoglobin subunit β	6.75	16102	191
2477	TRY3_HUMAN	Trypsin-3 precursor	7.46	33276	114
1062	K2C1_HUMAN	Keratin, type II cytoskeletal 1	8.16	66149	247
2360	ACTN1_HUMAN	α-Actinin-1	5.25	103563	185
1687	HBB_HUMAN	Hemoglobin subunit β	6.75	16102	165
17	PNPH_HUMAN	Purine nucleoside phosphorylase	6.45	32325	187
49	RCN3_HUMAN	Reticulocalbin-3	4.74	37470	175
848	ALBU_HUMAN	Serum albumin precursor	5.92	71317	135
2004	HBB_HUMAN	Hemoglobin subunit β	6.75	16102	152
1546	PSME1_HUMAN	Proteasome activator complex subunit 1	5.78	28876	133
1502	CNDP2_HUMAN	CNDP dipeptidase 2	5.66	53187	435
26	VIME_HUMAN	Vimentin	5.06	53676	887
1990	HSP71_HUMAN	Heat shock 70 kDa protein 1	5.48	70294	590
323	ENOA_HUMAN	α-Enolase	7.01	47481	260
2459	ENOA_HUMAN	α-Enolase	7.01	47481	316
120	NDUS1_HUMAN	NADH-ubiquinone oxidoreductase	5.89	80443	94
1720	HSP7C_HUMAN	Heat shock cognate 71 kDa protein	5.37	71082	957
575	GRP75_HUMAN	Stress-70 protein	5.87	73920	372
1565	HSP7C_HUMAN	Heat shock cognate 71 kDa protein	5.37	71082	346
1862	TRY3_HUMAN	Trypsin-3 precursor	7.46	33276	97
53	RSSA_HUMAN	40S ribosomal protein SA	4.79	32947	462
831	HBB_HUMAN	Hemoglobin subunit β	6.75	16102	126
2127	LMNA_HUMAN	Lamin-A/C	6.57	74380	185
1689	PRDX2_HUMAN	PRDX 2	5.66	22049	377
139	HPTR_HUMAN	Haptoglobin-related protein	6.42	39496	128
1656	TRY3_HUMAN	Trypsin-3 precursor	7.46	33276	134
1800	RD23A_HUMAN	UV excision repair protein RAD23	4.56	39642	277
265	EF1G_HUMAN	Elongation factor 1-γ	6.25	50429	329
2062	PRPS2_HUMAN	Ribose-phosphate pyrophosphokinase II	6.15	35146	159
269	HBB_HUMAN	Hemoglobin subunit β	6.75	16102	270
2345	TBB2C_HUMAN	Tubulin β-2C chain	4.79	50255	446
2012	IF4A1_HUMAN	Eukaryotic initiation factor 4A-I	5.32	46353	914
94	ATPB_HUMAN	ATP synthase subunit β	5.26	56525	220
13	TPD54_HUMAN	Tumor protein D54	5.26	22281	346
455	ALBU_HUMAN	Serum albumin	5.92	71317	166
64	NSF_HUMAN	Vesicle-fusing ATPase	6.52	83021	355
1749	LUM_HUMAN	Lumican	6.16	38747	144
2324	TBB5_HUMAN	Tubulin β-chain	4.78	50095	815
98	TBAK_HUMAN	Tubulin α-ubiquitous chain	4.94	50804	142
1833	DSG1_HUMAN	Desmoglein-1	4.9	114670	334
2346	TBB2A_HUMAN	Tubulin β-2A chain	4.78	50274	372
36	ALBU_HUMAN	Serum albumin	5.92	71317	236
2300	TPD54_HUMAN	Tumor protein D54	5.26	22281	288
295	2AAA_HUMAN	PR65-α isoform	5	66065	263
341	RD23A_HUMAN	UV excision repair protein RAD23	4.56	39642	146
2326	CAH1_HUMAN	Carbonic anhydrase 1	6.59	28909	545
209	HPTR_HUMAN	Haptoglobin-related protein	6.42	39496	149
308	CAH1_HUMAN	Carbonic anhydrase 1	6.59	28909	516
2303	CO3A1_HUMAN	Collagen α-1	6.21	139733	164

a) Spot numbers refer to Figs. 1C and 2.
b) Proteins were derived from Swiss-Prot and the National Center for Biotechnology Information nonredundant databases.
c) Theoretical p*I* and molecular weight obtained from Swiss-Prot.
d) MASCOT score for the identified proteins based on the peptide ion score.

Clinical Relevance

In OS, a novel chemotherapy responsiveness modality has long been desired to select the patients that would benefit from appropriate chemotherapy treatment. We performed a proteomic study using incisionally biopsied samples prior to treatment. A comparative protein expression study in 12 patients identified PRDX 2 as a novel chemotherapy responsiveness biomarker candidate. A subsequent Western blotting study on a further four cases established the correlation between higher PRDX 2 expression and poor response for chemotherapy.

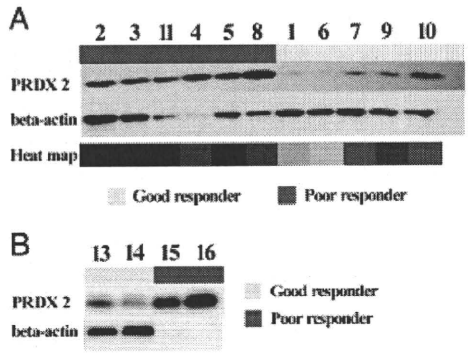

Figure 3. Western blotting for PRDX 2 with specific antibodies. (A) The eleven OS biopsy samples those were used in proteomic analysis. The amount of protein sample 12 in Figs. 1 and 2 was not enough for SDS-PAGE/Western blotting. The heat map under the Western blotting panel was derived from Fig. 2. (B) The additional four OS samples.

Many of the parameters characterizing OS such as the age of onset, its histological presentation, and clinical behavior may vary widely between cases [26, 27], and this variation makes the identification of novel predictive biomarkers difficult. In this study, we tried to compensate for this variation by selecting cases that had almost identical clinical background.

PRDX 2 was included in the 21 proteins found to be upregulated in poor responders. PRDX 2 overexpression has been related to chemoresistance or radioresistance in breast, head and neck, gastric carcinomas, and Molt-4 leukemia [28–30]. However, the association of PRDX 2 expression with OS has not been reported previously. This may be due to discordance between mRNA and protein expression, the fact that a different patient population was studied, or the fact that transcriptome and proteome studies cannot uncover the entire genome data. These results, therefore, also suggest that studies using proteomic tools are able to reveal unique molecular aspects of OS.

From this study, PRDX 2 expression may be used as a novel chemotherapy responsiveness biomarker of patients with OS. PRDX 2 expression as measured using Western blotting correlated positively with poor response to chemotherapy. The predictive value of PRDX 2 expression was validated in an additional four OS samples. Applying these findings in a clinical setting poses the next challenge. One advantage is that, as the incisional biopsy is a procedure performed routinely in establishing the diagnosis in OS, the examination of PRDX 2 expression can be performed without any additional invasive examinations, suing material obtained during the biopsy procedure.

PRDX 2 has been previously known as a natural killer-enhancing factor B [31]. It is induced by various oxidative stimuli and plays an important protective role from oxidative radical damage by reactive oxygen species and reactive nitrogen species [32–34]. PRDX 2 over-expressing cells are more resistant to the oxidative damage caused by H_2O_2, *t-butyl*-hydroperoxide, and methyl mercury [35]. Its expression is correlated with resistance to apoptosis induced by radiation therapy or anticancer drugs [25, 36], highlighting the potential clinical importance of PRDX 2 in chemotherapy resistance in cancer. However, the functional role of PRDX 2 in chemotherapy responsiveness in OS is still unclear. Conducting further basic research on the function of PRDX 2 will pave the way for further understanding of the molecular background of OS and, hopefully, for novel diagnostic and therapeutic applications.

The localization of PRDX2 should be examined by immunohistochemical studies. We used homogenized tissues for protein expression study, and the localization of PRDX2 was not cleared yet. PRDX2 is the third most abundant protein in erythrocytes, and the two most abundant ones, hemoglobin and carbonic anhydrase, and serum albumin were also identified in the tumor tissues (Fig. 2, Table 2). The differential expression level of some proteins might be due to the difference in vascularity in the tumor tissues. The antibody against PRDX2 for Western blotting in this study was not applicable for immunohistochemistry (data not shown). To address this issue, we are developing monoclonal antibody for immunohistochemistry.

In conclusion, global protein expression profiling revealed the proteomic background of OS and identified novel associations of a number of proteins with chemotherapy responsiveness of OS. Of the proteins the expression of which was shown to have predictive values, we successfully validated the association of PRDX 2 expression with poor responsiveness to chemotherapy. The expression of the other proteins may still have predictive value and further validation studies may prove it. Evaluation of PRDX 2 expression may allow the identification of poor responsiveness prior to the initiation of chemotherapy in OS patients who may thus benefit from highly effective treatment in the future. The further extensive validation studies on PRDX 2 including more number of cases will establish

the novel prognostic modalities to optimize the therapeutic strategy.

In our study, PRDX 2 was identified as a novel candidate chemotherapy responsiveness biomarker through the use of modern global protein expression modalities. Our study suggests the possible use of PRDX 2 expression for personalized medicine for OS patients.

This study was supported in part by foundation for Promotion of Cancer Research in Japan. The authors appreciate the excellent technical support of Yukiko Fujie in the Western blotting experiments.

The authors have declared no conflict of interest.

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