

Proteomic Study of Sera from Patients with Bladder Cancer: Usefulness of S100A8 and S100A9 Proteins

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Abstract. To effectively obtain tumour-specific markers, fractionated proteins obtained using reversed-phase high-performance liquid chromatography for patient-matched pre- and postoperative sera from bladder cancer patients were compared by two-dimensional gel electrophoresis. The usefulness of the identified proteins was confirmed immunohistochemically. S100A8 and S100A9 were identified as tumour-associated proteins. The increased immunoreactive expression of S100A8 protein was associated with bladder wall muscle invasion of the tumour and cancer-specific survival ($p < 0.05$), and the increased immunoreactive expression of S100A9 protein was associated with the tumour grade ($p < 0.05$). In addition, increased expressions of both proteins was associated with recurrence-free survival at a median follow-up of 32.9 months (both $p < 0.05$). On multivariate analysis, the expression of S100A8 was a significant predictor of recurrence ($p < 0.05$). These findings may help to identify biologically aggressive tumors and, thus, patients who might benefit from more intensive adjuvant therapy.

Bladder cancer, responsible for an estimated 14,100 deaths in the USA alone in 2008, as well as 68,810 expected new cases, is the fifth most common cancer in men and women in the United States (1). Urothelial carcinoma (UC) represents over 90% of all bladder carcinoma, and approximately 75% of these are papillary tumours localized in the urothelium

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(superficial, pTa) or lamina propria (pT1). Even in pTa tumours, about 70% of cases recur even after transurethral resection, 30-50% of which progress to higher-grade tumours (2). In tumours initially presenting with bladder muscle invasion, 50% of patients treated endoscopically show recurrence within 2 years after treatment (3), and two-thirds of patients die within 5 years (4). In addition, patient outcome is strongly correlated with the tumour stage at the initial diagnosis. The development of new, highly effective and accurate screening tests that could detect most tumors even at an early stage, before metastasis has occurred, might markedly decrease cancer death rates (5).

The traditional diagnostic methods involving urine cytology or cystoscopy achieve a specificity of 90-100%, but they suffer from a lower sensitivity, particularly in the detection of low-grade tumours (6). New noninvasive urine assays employing NMP22 BladderCheck[®] (Inverness Medical Innovations Inc., MA, USA) or BTastat[®] (POLYMEDCO Inc., NY, USA) have shown improved detection rates of bladder cancer, but they cannot match the usefulness of cytology, especially in the case of superficial tumours (7, 8). Indeed, no single protein marker has been reported to show sufficient sensitivity and specificity in order to predict the patient outcome and tumour presence. Gene expression microarray profiling is being used to highlight novel targets as well as pattern-based prognosis (9-11), but gene expression alone cannot provide sufficient information for deciphering a pathological phenotype, the key to the understanding of which lies at the protein level in cancer (12). Proteomics has become increasingly recognised as a new useful tool for the identification of therapeutic targets, diagnostic methods, and prediction of drug effectiveness (13, 14). Proteomic patterns of body fluids present new opportunities for the development of novel, highly sensitive diagnostic tools for the early detection of cancer (15). Serum is expected to be an excellent source of protein biomarkers

because it circulates through, or comes into contact with, all tissues, where it is likely to pick up proteins secreted or shed by tissues (16).

By analysing proteomic patterns of pre- and postoperative mixed sera from 2 bladder cancer patients, the present study was conducted to identify altered expressions of proteins and to evaluate the usefulness of this data by immunohistochemical analysis of 77 surgically resected cases of bladder cancer.

Tumour-associated proteins in patients with bladder cancer were analysed using reversed-phase high-performance liquid chromatography (RP-HPLC) and two-dimensional gel electrophoresis (2-DE)/mass spectrometry (MS). The calcium-binding proteins S100A8 (calgranulin-A, MRP-8) and S100A9 (calgranulin-B, MRP-14) showed a decrease in postoperative sera. It was immunohistochemically confirmed that the expression pattern of these proteins was related to the tumour stage, grade, and prognosis of the patients.

Materials and Methods

Samples. Pre- and postoperative sera from two patients with bladder cancer, both pT3aN0M0 UCs, were collected at Kitasato University Hospital. The 2002 TNM and WHO classifications were used for the determination of the pathological stage and grade of the tumour (17). For each patient, preoperative sera were obtained at the initial diagnosis and postoperative sera were from matched patients 3–5 weeks after surgery.

Seventy-seven formalin-fixed and paraffin-embedded UCs of the bladder, which were surgically resected at Kitasato University Hospital from 1990 to 2004, were used in this study. Ten non-neoplastic bladder tissues were also investigated. The median follow-up was 32.9 months for patients alive at the last follow-up visit. When patients died, the cause of death was determined by the attending physicians.

This study was approved by the Ethics Committee of Kitasato University School of Medicine. All patients were informed of the aim of the study and gave consent for the use of their samples.

Depletion of albumin/IgG and ultrafiltration. Fifty μ l of each serum were tested for the depletion of high-abundant albumin and IgG using ProteoExtract Albumin/IgG Removal Kit (EMD Chemicals Inc., Darmstadt, Germany) according to the manufacturer's instructions, and the flow-through fraction was collected.

Using an ultrafiltration spin column, Vivaspin 2 (PES, MWCO: 10 kDa, Sartorius AG, Goettingen, Germany), flow-through fractions were concentrated using a cutoff below a molecular weight of 10 kDa according to the manufacturer's instructions. To remove salts, 1 ml of 0.1% trifluoroacetic acid (TFA; Kokusan Chemical, Tokyo, Japan) /H₂O was added and samples were spun again until a final sample volume of 100 μ l was obtained. Finally, samples were placed in collection tubes and stored at –80°C until use.

HPLC sample fractionation. HPLC-grade acetonitrile (ACN) and H₂O were purchased from Nacalai Tesque (Kyoto, Japan). Concentrated flow-through samples were separated by HPLC (Nanospace SI-2; Shiseido Fine Chemicals, Tokyo, Japan). Separations were conducted employing a reversed-phase (RP)

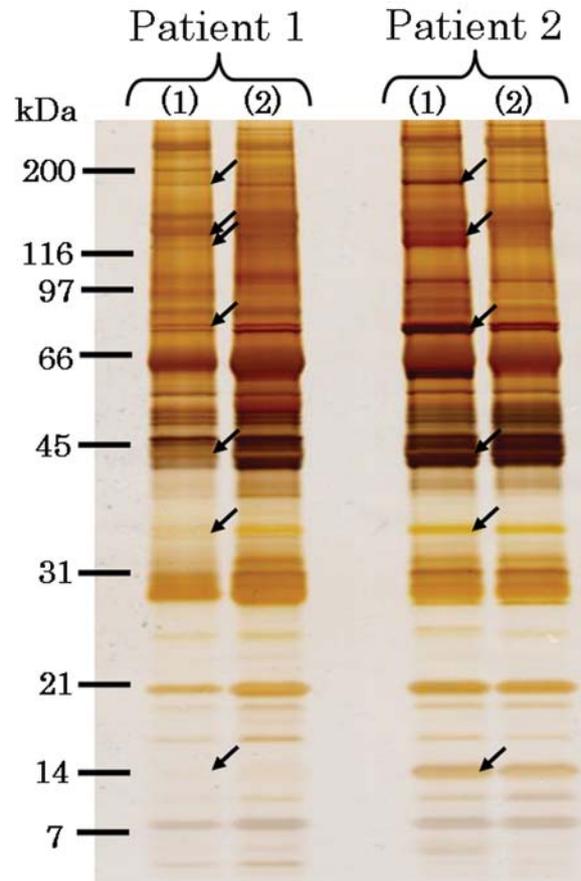


Figure 1. Comparison of protein patterns in pre- (1) and postoperative (2) sera of the 16th fraction. Protein patterns of each patient's sera were similar, but some altered subtle spots were confirmed (\leftarrow).

column (Intrada WP-RP 3.0 mm ID \times 150 mm; Imtakt, Kyoto, Japan, using an 80 μ l injection of each concentrated flow-through sample at room temperature (R/T). The RP separations for each sample were performed under a set of predetermined conditions using a multi-segment elution gradient, with eluent A (0.1% TFA/99.9% H₂O) and eluent B (90% ACN/0.08% TFA/9.92% H₂O). The gradient conditions consisted of three steps with increasing concentrations of eluent B: 5–51% B for 23 min, 51–95% B for 1 min, and held at 95% B for 10 min at a flow rate of 0.4 ml/min for a total run time of 65 min. For consecutive runs, the same gradient conditions were set up to re-equilibrate the column.

The chromatograms were monitored at 218 nm, and 40 fractions were collected at 0.5 min intervals from 19.0 to 39.0 min. Then, 200- μ l aliquots of all fractions were separated into 25 and 175- μ l fractions and stored at –80°C for subsequent sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and 2-DE analysis, respectively.

SDS-PAGE. All 25- μ l fractions were lyophilized before use, and these were solubilized in 10 μ l of rehydrate buffer containing 50 mM Tris-HCl (pH 6.8), 50 mM dithiothreitol (DTT), 0.5% SDS, 0.02% bromophenol blue (BPB), and 10% glycerol at R/T for 1 h

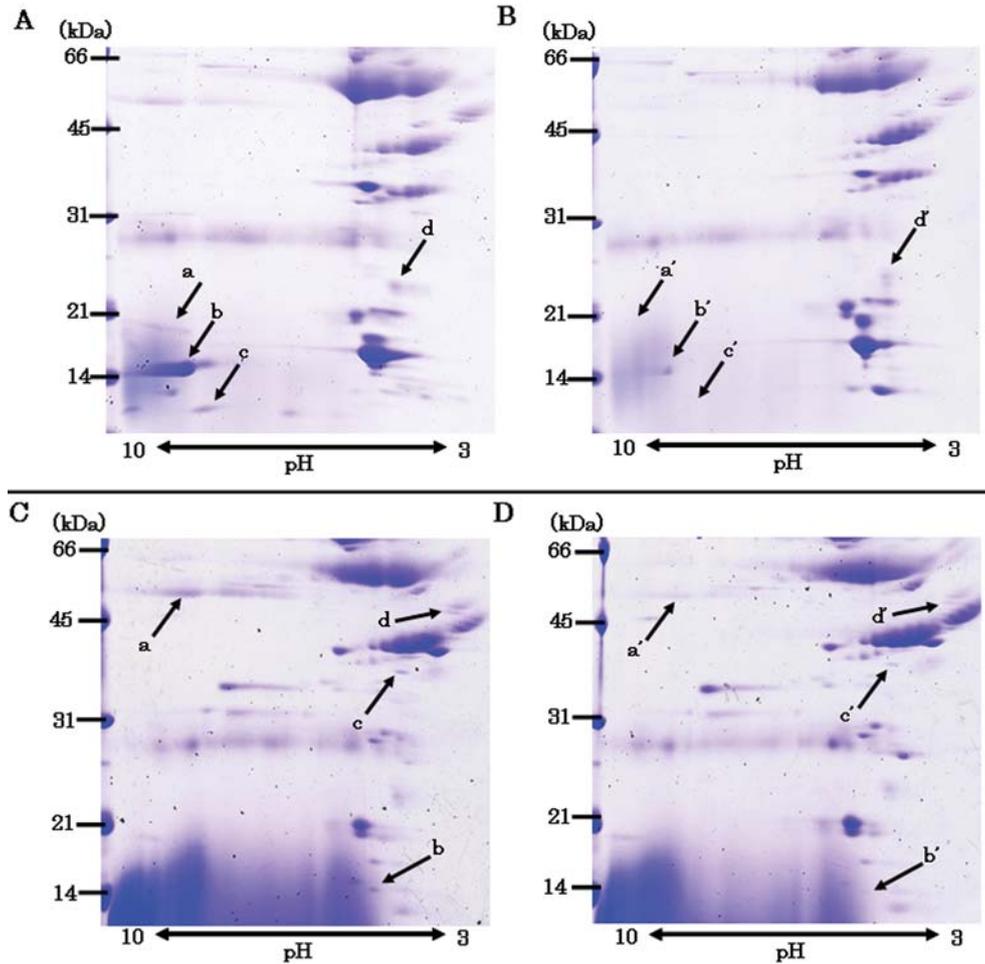


Figure 2. 2-DE protein pattern of fractionated mixed sera of the 13th (A, B) and 16th (C, D) fractions. Comparing the protein pattern of pre- (A, C) with matched postoperative sera (B, D), decreased levels of protein spots were identified in each fraction.

and applied to 10-20% acrylamide gels (Perfect NT gel W; DRC, Kanagawa, Japan). Electrophoresis was carried out at a constant voltage of 150 V at R/T in SDS-PAGE buffer (0.3% Tris-base, 1.4% Glycine, 0.1% SDS) until the BPB front dropped out of the bottom of the gel. Finally, gels were stained with 2D-Silver Stain Reagent (Cosmo Bio, Tokyo, Japan) according to the manufacturer's instructions.

2-DE gel electrophoresis. One-hundred and seventy-five μ l of each fraction from the pre- and postoperative sera of the two patients, were mixed, as described above. Lyophilized samples were solubilized in 70 μ l of rehydrate buffer containing 7 M urea, 2 M thiourea, 2% CHAPS, 10 mM DTT, 2.5% pH 3–10 Pharmalyte (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA), and 1 tablet/dl of complete mini EDTA-free protease inhibitors (Roche Diagnostics, Mannheim, Germany) by mixing at R/T for 1 h. The Agarose-2-DE method used in this study was previously described by Oh-Ishi *et al.* with some modifications to make it suitable for mini-gel (18, 19). The first-dimensional agarose isoelectric focusing gel (IEF) gel (2.0 mm in inner diameter and 120 mm in length) was made by single Pharmalyte pH 3-10 (GE Healthcare Bio-Sciences

Corp.). Each fractionated rehydrated protein sample was applied at the cathodic end of the agarose IEF gel, and loaded at constant voltage of 400 V for 15 h at 4°C. After being fixed in 10% (v/v) trichloroacetic acid and 5% (w/v) sulfosalicylic acid for 5 min at R/T with mild shaking, the agarose gel was placed in distilled water for 10 min at R/T. The agarose gel was then placed on the top of the second-dimensional 10-20% acrylamide gradient gel (Perfect NT gel W, DRC) and electrophoresed at a constant voltage of 150 V in SDS-PAGE buffer at R/T. Finally, gels were stained by Coomassie brilliant blue R-350 (CBB) (PhastGel Blue R; Amersham Pharmacia Biotech AB, Uppsala, Sweden).

In-gel digestion /identification by LC-MS/MS. Protein spots were excised from the gel, which were destained with 50% (v/v) ACN/50 mM NH_4HCO_3 until opaque and colorless, dehydrated with 100% (v/v) ACN, and dried under vacuum conditions. The gel pieces were rehydrated in 7 μ l of trypsin solution containing 50 ng/ μ l of proteomics sequencing grade modified trypsin (Roche Diagnostics) at 4°C for 45 min and incubated at 37°C for 24 h with a minimalized volume of trypsin solution and 8 μ l of 50 mM Tris-HCl buffer (pH 9.0). After incubation, the digested protein

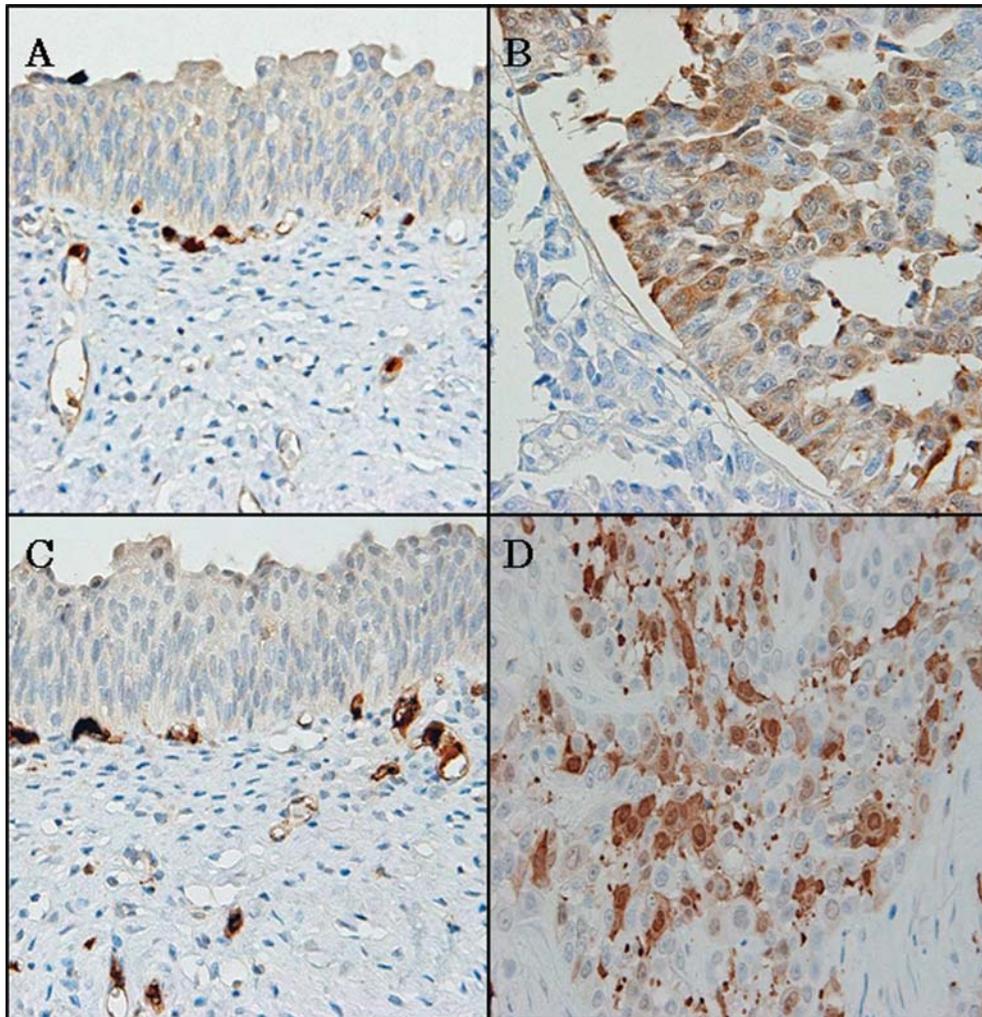


Figure 3. Immunohistochemical staining of UCs for S100A8 (A, B) and S100A9 (C, D). No clear staining of S100A8 (A) and S100A9 (C) was observed in normal urothelial tissue. In UCs, S100A8 (B) and S100A9 (D) were observed in tumour cells.

fragments eluted in solutions were collected and the gel was washed once in 8 μ l of 5% formic acid/50% ACN and collected in the same tube. Solutions containing the protein fragment were identified using the LC-MS/MS system, which consisted of an HPLC system (Nanospace SI-2; Shiseido Fine Chemicals) and ion-trap mass spectroscopy (LCQ Deca; Thermo Fisher Scientific, MA, USA).

The SEQUEST program (<http://fields.scripps.edu/sequest/index.html>) was used to identify proteins from measured masses of the trypsin digestions and their MS/MS fragments.

Immunohistochemical staining (IHC). Seventy-seven cases of paraffin-embedded UCs and normal bladder tissue sections were used for the immunohistochemical study. The sections were deparaffinized in xylene, rehydrated in a descending ethanol series, and then treated with 3% hydrogen peroxide for 10 min. The sections were continuously antigen-retrieved in 0.01 M citrate buffer (pH 6.0)/0.1% Tween 20 at 121°C for 10 min. After blocking with 0.5% casein at R/T for 10 min, the sections were reacted with a 1:400 dilution of each of anti-S100A8 and anti-S100A9 (both

Santa Cruz Biotechnology, CA, USA), diluted with 2% normal swine serum (DAKO, Glostrup, Denmark)/Tris-buffered saline (TBS; pH 7.4), at R/T for 2 h. The sections were then reacted with 1:200 diluted biotinylated anti-goat IgG (Vector Laboratories, Burlingame, CA, USA) and streptavidin-HRP (LSAB2 kit/HRP; DAKO) at R/T for 30 min each. The sections were rinsed in TBS three times for 5 min each and used immediately after every reaction. Finally, the sections were visualized using Stable DAB solution (Invitrogen, Carlsbad, CA, USA) and counterstained with Mayer's haematoxylin.

Evaluation of IHC. IHC was scored semiquantitatively by incorporating both the staining intensity and percentage of positive cells (labeling frequency). The percentage of positive tumour cells was scored as 0 (0%), 1+ (1-25%), 2+ (26-50%), 3+ (51-75%), or 4+ (76-100%). The staining intensity was also scored as 1+ (weakly positive), 2+ (moderately positive), or 3+ (strongly positive). The sum index was obtained by totaling the staining intensity and percentage scores (20).

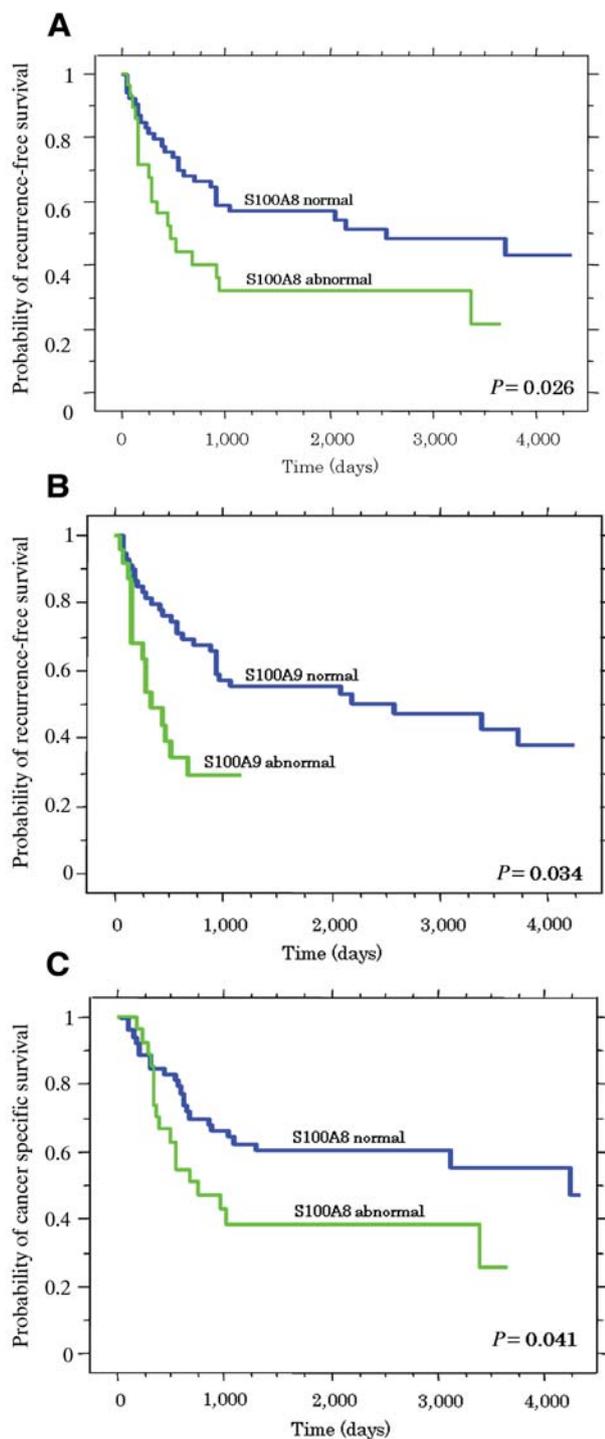


Figure 4. The probability of recurrence-free survival after radical cystectomy according to expressions of S100A8 (A) and S100A9 (B), and cancer-specific survival based on the expression of S100A8 (C).

In a preliminary study, the discriminative value for bladder cancer characteristics and prognosis was assessed using each value in the sum index as the cutoff point. Kaplan-Meier analyses revealed that a cut-off sum index value (score 2 in S100A8 and

score 4 in S100A9) provided the best positive and negative prediction for bladder cancer progression and survival (data not shown). Likewise, evaluation of the association of each cutoff value for S100A8 and S100A9 with clinical, pathological, and molecular characteristics showed that score 2 in S100A8 and 4 in S100A9 were significant (data not shown). The sum index values for these proteins were further stratified into normal (scores 0 to 2 in S100A8 and 0 to 4 in S100A9) and abnormal (scores 3 or greater in S100A8 and 5 or greater in S100A9) for the purposes of presentation.

Statistical analysis. Statistical differences among gender, pathologic stage, pathologic grade, presence of carcinoma *in situ*, lymph node status, and lymphovascular invasion were assessed by employing Fisher's exact test. Patient survival was calculated using the Kaplan-Meier method. The log-rank test was applied to assess the significance of differences in the survival rate. Multivariate analyses were performed by employing the Cox proportional hazards regression model. $p < 0.05$ was considered significant. All reported p -values were generated by two-sided tests. All analyses were performed using the Statistical Package for Social Sciences, version 11.0, for Windows (SPSS, Chicago, IL, USA).

Results

Reproducibility of fractionation. SDS-PAGE was performed for all fractionated samples to compare the protein patterns. Electrophoresis was carried out in each fraction eluted from the column at the same time. For example, protein patterns of the 16th fraction are presented in Figure 1. Protein patterns in each fraction of pre-operative sera and these of post-operative sera were similar from the same patient.

Decreased expression of proteins in postoperative sera. To determine the proteins that decreased in the post-operative in comparison with the pre-operative sera of patients with bladder cancer, 2-DE. Electrophoresis was carried out with the same pre- and post-operative fractions from each patient (Figure 2). Protein spots were visualized by CBB staining.

In this study, 68 protein spots decreased in the post-operative compared to the preoperative sera, and 54 of these spots were identified by LC-MS/MS analysis. Four spots were identified from the 13th fraction and 4 spots from the 16th fraction that contained S100A8 and S100A9 proteins (Figure 2, Table I).

Association of S100A8 and S100A9 expressions with clinicopathological characteristics. To evaluate the utility of S100A8 and S100A9 proteins as diagnostic markers, an IHC study was performed involving UCs and their corresponding normal bladder tissues. The expression of both S100A8 and S100A9 proteins was observed in inflammatory cells such as neutrophils and macrophages, but not in normal bladder epithelial cells (Figures 3A and C). In UCs, expressions of S100A8 and S100A9 were observed in tumour cells at various levels from case to case (Figures 3B and D).

Table I. Identified proteins whose expression decreased in postoperative sera in (A) 13th fraction and (B) 16th fraction.

A.							
Fraction	No.	Protein name	Gene name	Score	Sequence coverage (%)	Sequence coverage (%)	Accession number
13	a	Hemoglobin subunit beta	HBB	60.2	20.1	20.1	P68871
13	b	Hemoglobin subunit delta	HBD	80.3	64.2	64.2	P02042
13	c	S100 calcium-binding protein A8	S100A8	156.2	58.7	58.7	P05109
13	d	Plasma retinol-binding protein [Precursor]	RBP4	76.1	16.3	16.3	P07253
B.							
Fraction	No.	Protein name	Gene name	Score	Sequence coverage (%)	Sequenced peptide	Accession number
16	a	Ig alpha-1 chain C region	IGHA1	96.2	22.26	10	P01876
16	b	S100 calcium-binding proteinA9	S100A9	90.2	60.32	9	P06702
16	c	Gelsolin [Precursor]	GSN	70.2	7.64	7	P06396
16	d	alpha-2-HS-glycoprotein	AHSG	80.3	12.26	8	P02765

Table II. Association of S100A8 and S100A9 expression with clinical and pathologic characteristics of patients who underwent radical cystectomy for bladder TCC.

	No. of pts.	S100A8 expression			S100A9 expression		
		Normal	Abnormal	p-Value*	Normal	Abnormal	p-Value*
Total (%)	77	53 (68.8)	24 (31.2)		58 (75.3)	19 (24.7)	<0.0001
Gender (%)							<0.034
Male	63 (81.8)	44 (69.8)	19 (30.2)		51 (81.0)	12 (19.0)	
Female	14 (18.2)	10 (71.4)	4 (28.6)	N.S.	7 (50.0)	7 (50.0)	
Pathological stage (%)							N.S.
Pa, Pis, P1	17 (22.1)	16 (94.1)	1 (5.9)		16 (94.1)	1 (5.9)	
P2-P4	60 (77.9)	37 (61.7)	23 (38.3)	<0.015	42 (70.0)	18 (30.0)	
Pathological grade (%)							< 0.036
Grades 1 and 2	37 (48.1)	29 (78.4)	8 (21.6)		32 (86.5)	5 (13.5)	
Grade 3	40 (51.9)	24 (60.0)	16 (40.0)	N.S.	26 (65)	14 (35.0)	
Carcinoma <i>in situ</i> (%)							N.S.
Negative	64 (83.1)	43 (67.2)	21 (32.8)		48 (75.0)	16 (25.0)	
Positive	13 (16.9)	10 (76.9)	3 (23.1)	N.S.	10 (76.9)	3 (23.1)	
Lymph node status (%)**							N.S.
N0	54 (70.1)	41 (75.9)	13 (24.1)		44 (81.5)	10 (18.5)	
N1, N2	18 (23.4)	10 (55.6)	8 (44.4)	N.S.	11 (57.9)	7 (38.9)	
Lymphovascular invasion (%)***							N.S.
Negative	27 (35.1)	20 (74.1)	7 (25.9)		22 (81.5)	5 (18.5)	
Positive	41 (53.2)	26 (63.4)	15 (36.6)	N.S.	28 (68.3)	13 (31.7)	

*Fisher's exact test (two-sided); **five patients had unknown pathological status of lymph node; ***nine patients had no lymphovascular status; Pts., patients; N.S., not significant.

Table III. Multivariate Cox proportional hazards regression analyses of the expression of S100A8/S100A9 and clinicopathological findings for predicting clinical outcome after radical cystectomy.

	Cancer recurrence			Cancer-specific mortality		
	Odds ratio	95%CI	p-Value	Odds ratio	95%CI	p-Value
S100A8	2.34	1.01-5.39	0.04	2.19	0.94-5.12	N.S
S100A9	0.42	0.15-1.17	N.S	0.44	0.15-1.23	N.S
Pathological stage	1.57	1.06-2.31	0.03	1.7	1.14-2.56	0.01
Pathological grade	1.59	0.77-3.26	N.S	1.52	0.73-3.13	N.S
Lymphovascular invasion	1.23	0.55-2.74	N.S	1.72	0.79-3.69	N.S
Lymph node metastasis	1.59	1.07-2.36	0.02	1.41	0.95-2.09	N.S

N.S., Not significant.

Abnormal expressions of S100A8 and S100A9 proteins were observed in 24 (31.2%) and 19 (24.7%) of the 77 UCs, respectively. A significant relationship was observed between the expression of S100A8 and S100A9 proteins ($p < 0.0001$). The relationship between the clinicopathological characteristics of UC patients and S100A8 and S100A9 expressions is presented in Table II. The abnormal expression of S100A8 protein was significantly higher in tumour stage pT2-4 than in pTa, pTis, and pT1 cases ($p < 0.05$). The abnormal expression of S100A9 was significantly higher in females than in males, and in the tumour grade G3 than in G1 of G2 ($p < 0.0001$ and $p < 0.05$, respectively). The presence of carcinoma *in situ*, lymph node metastases, and lymphovascular invasion did not differ significantly between phenotypes with normal and abnormal expressions of either S100A8 or S100A9.

Association of S100A8 and S100A9 expressions with clinical outcomes. At a median follow-up of 32.9 months, a Kaplan-Meier projection indicated that the expression of both S100A8 and S100A9 were significantly associated with recurrence-free survival ($p < 0.05$; Figure 4). In addition, the expression of S100A8 was associated with cancer-specific death ($p < 0.05$; Figure 4). In Cox proportional hazards regression analyses, S100A8 expression, the pathological stage and lymph node metastasis were significantly associated with recurrence-free survival ($p < 0.05$; Table III). The expression of S100A8 showed the greatest odds ratio for recurrence-free survival ($p < 0.05$; Table III, odds ratio: 2.34, 95% confidence interval: 1.01 to 5.39). However, only the pathological stage was found to be strongly associated with cancer-specific survival on Cox proportional hazards regression analyses.

Discussion

S100A8 and S100A9 were decreased in postoperative compared with preoperative sera from patients with UCs. These proteins have also been implicated in a variety of chronic inflammatory conditions such as cystic fibrosis,

rheumatoid arthritis, and transplant rejection (21). However, several S100 proteins, including S100A8 and S100A9, have received much attention concerning their possible role in tumour development and progression (22-24), and many studies have reported an increased expression of S100A8 or S100A9 protein in a variety of epithelial tumours, including gastric, ovarian, colon, and prostate cancer (25-30). In addition, increased expressions of both proteins in cancer patient sera were also reported in ovarian and colorectal cancers (28, 29). Therefore, IHC was performed to confirm whether the identified S100A8 and S100A9 proteins in sera originated from bladder tumours, and the relation between the expression of both proteins and clinicopathological factors in UCs.

In this study, the immunohistochemical analysis revealed that the expression levels of S100A8 and S100A9 proteins were significantly correlated with cancer recurrence-free survival. This finding may be attributable to the structure of S100A8 and S100A9 proteins, consisting of heterocomplexes of each other (31). Furthermore, the rate of abnormal expression of S100A8 protein in cases with bladder wall muscle invasion was significantly higher than those without invasion (Table II), and the rate of abnormal expression of S100A9 protein in high-grade was also significantly higher than in low-grade tumours (Table II).

It is noteworthy that analysis of the clinical follow-up data of UC patients revealed a significant association of S100A8 and S100A9 expressions with the clinical outcome. This finding indicates that abnormal S100A8 and S100A9 expressions are of potential clinical significance in making a prognosis, and corresponding to a report whereby abnormal expression with other members of S100 family proteins, S100A4 and S100A2, indicates a substantially increased risk of disease progression and cancer-specific death (20).

Secreted proteins can be analysed by relatively noninvasive techniques and, therefore, have the potential to greatly enhance screening implementation and acceptance. The discovery of S100A8 and S100A9 as serological

markers could have a marked impact in the prediction of the clinical outcome of bladder cancer patients. Kim *et al.* (29) compared serum levels of S100A8 and S100A9 proteins in colorectal cancer and inflammatory conditions, and identified elevated levels of both proteins in some patients with inflammatory conditions. These data indicate that neither protein could be immediately used as the sole biomarker for the diagnosis of bladder cancer. However, this is also true for most, if not all, serum markers for cancer, and so the application of multiple biomarkers is generally considered preferable to increase the sensitivity and specificity of diagnosis (32, 33). Therefore, it is suggested that a diagnosis employing S100A8 and S100A9 expressions combined with other S100 family proteins for bladder cancer may lead to additional new biomarkers helpful for identifying more biologically aggressive cancer and predicting the clinical outcome of bladder cancer patients (20).

Conclusion

A proteomic analysis of sera from bladder cancer patients was performed. Comparing protein patterns in post-compared with preoperative sera, altered expressions of S100A8 and S100A9 proteins were identified. Using antibodies against S100A8 and S100A9 proteins, an immunohistochemical study revealed that the presence of high levels of S100A8 and S100A9 proteins in UCs is associated with the pathological grade and stage, respectively.

In addition, on analysing the clinical follow-up data of UCs patients, it was revealed that abnormal expressions of S100A8 or S100A9 proteins are correlated with a poor prognosis. Thus, serum proteomics and immunohistochemical studies on the expressions of S100A8 and S100A9 proteins may aid in identifying more biologically aggressive cancers and patients who might benefit most from interventional therapy.

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