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High expression of heat shock protein 105 predicts a favorable prognosis for patients with urinary bladder cancer treated with radical cystectomy

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Abstract. Heat shock protein 105 (Hsp105) is one of the cancer/testis antigens, which is overexpressed in a variety of cancer cells, including urinary bladder cancer, and has been investigated as a target molecule for immunotherapy due to its immunogenicity. In this study, we assessed the expression of Hsp105 in primary bladder cancer samples from 84 patients treated with radical cystectomy, using immunohistochemical analysis, and investigated its correlation with clinicopathological characteristics and cancer-specific survival. The immunoreactivity of Hsp105 expression was evaluated as a score of 0-3, according to the intensity of the signal. The Hsp105 expression was high (score 2 or 3) in 31 cases and low (score 0 or 1) in 53 cases; however, it was not significantly correlated with age, nuclear grade, pathological tumor stage and previous intravesical Bacillus Calmette-Guérin immunotherapy. Female gender, lymphovascular invasion and lymph node metastasis were associated with low Hsp105 scores, although the differences were not statistically significant (P=0.071, 0.061 and 0.175, respectively). However, a high Hsp105 score was significantly associated with a favorable prognosis (P=0.017) and was identified as an independent prognostic factor by multivariate analysis (P=0.032; hazard ratio, 2.34). These findings suggested that the expression of Hsp105 may be a novel indicator of a favorable prognosis in bladder cancer.

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Introduction

Urinary bladder cancer is the second most common urological cancer and is responsible for 2.0% of cancer-related mortality cases worldwide (1). Muscle invasion is a key factor for the prognosis of patients with bladder cancer. Non-muscle invasive bladder cancer (stages Ta, T1 and Tis) generally has a good prognosis, whereas muscle-invasive bladder cancer (MIBC; stages T2, T3 and T4) frequently develops metastases and has a poor prognosis, with a 5-year survival of 65-70% following radical cystectomy (2,3). However, clinical and pathological markers for the prediction of prognosis of patients with bladder cancer following radical cystectomy have not yet been established.

Heat shock proteins (HSPs) are stress proteins released in response to various stress factors, such as heat, infection, ischemia and cancer (4). The expression of HSPs in cancer cells has been implicated in the regulation of apoptosis (5,6). HSPs also modulate cancer cell immunogenicity (7,8). Heat shock protein 105 (Hsp105) is a high-molecular-weight protein that belongs to the Hsp105/110 family. It is one of several cancer/testis antigens that were identified by serological analysis of antigens by recombinant expression cloning (SEREX) (9). Previous studies have suggested that Hsp105 enhances stress-induced apoptosis in embryonal cells (10), while suppressing stress-induced apoptosis in neuronal (11) and cancer cells (12,13). Hosaka *et al* (12) reported that the knockdown of Hsp105 induced apoptosis in the HCT116 human colon cancer and the KATO-3 human gastric cancer cell lines. Furthermore, Hsp105 was shown to be overexpressed in a variety of human cancer cells, including colorectal, pancreatic, thyroid, esophageal, breast and bladder cancer cells (14). High expression of Hsp105 has been associated with advanced stage of squamous cell carcinoma of the tongue (15), in addition to advanced stage and poor prognosis of lung adenocarcinoma (16). Recently, Hsp105 was proposed as a target molecule for immunotherapy due to its immunogenicity (17). However, no correlation between the level of Hsp105 expression and the prognosis for bladder cancer has been reported thus far.

Table I. Correlation between Hsp105 score and clinicopathological characteristics in 84 primary bladder cancer cases.

Clinicopathological characteristics	n	Hsp105 score		P-value
		Low (0, 1)	High (2, 3)	
All cases	84	53 (63)	31 (37)	
Gender				
Male	70	41 (59)	29 (41)	0.071
Female	14	12 (86)	2 (14)	
Age (years)				
<65	44	29 (66)	15 (34)	0.575
>66	40	24 (60)	16 (40)	
Nuclear grade[a]				
G1/G2	21	13 (62)	8 (38)	0.809
G3	60	37 (62)	23 (38)	
Pathological tumor stage				
pTa/pTis/pT1	23	14 (61)	9 (39)	0.995
pT2/pT3/pT4	61	39 (64)	22 (36)	
Lymphovascular invasion				
Negative	35	18 (51)	17 (49)	0.061
Positive	49	35 (71)	14 (29)	
Lymph node metastasis				
pN0	65	38 (58)	27 (42)	0.175
pN1/pN2/pN3	19	15 (79)	4 (21)	
Previous BCG therapy				
Negative	63	40 (63)	23 (37)	0.896
Positive	21	13 (62)	8 (38)	

[a]Determined in 81 samples of urothelial carcinoma. P-values were calculated using the Pearson's χ^2 or Fisher's exact tests. Hsp105, heat shock protein 105; BCG, Bacillus Calmette-Guérin. Parenthetical data represent percentage values.

The aim of this study was to investigate Hsp105 expression in primary bladder cancer tissues from patients treated with radical cystectomy and its effect on cancer-specific survival (CSS) of bladder cancer.

Materials and methods

Surgical specimens. A tissue microarray (TMA) containing 88 human bladder specimens was used in this study. The bladder specimens included 84 primary bladder cancer samples (81 urothelial and 3 squamous cell carcinomas) and 4 specimens of non-cancerous bladder mucosa. The 84 primary bladder cancer samples were obtained from patients who underwent radical cystectomy at the University of Tokyo Hospital between 1990 and 2005. The patients comprised 70 males and 14 females, with a median age of 65 years (range, 39-81 years). The 4 non-cancerous bladder specimens were obtained from patients who underwent cystectomy for causes other than malignancy.

All patients provided consent for this study prior to surgery. All analyses of human materials were performed according to the guidelines of the Ethics Committee of the University of Tokyo. The samples were diagnosed and classified by a urological pathologist (T.M.) at the Department of Pathology, University of Tokyo Hospital.

Immunohistochemical analysis. Immunohistochemical staining was performed using the EnVision+ system HRP labelled polymer anti-rabbit kit (Dako, Glostrup, Denmark) according to the manufacturer's instructions. The primary antibody used was a rabbit polyclonal anti-human Hsp105 antibody (N-187: sc-6241), purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Following the visualization of Hsp105 using Liquid DAB+ substrate chromogen system (Dako), the sections were counterstained with hematoxylin. The immunoreactivity of Hsp105 expression was scored by a urological pathologist (T.M.) according to the intensity of the signal as follows: score 0, none; score 1, mild; score 2, moderate; and score 3, intense. Hsp105 scores of 0 and 1 were defined as 'low', whereas scores of 2 and 3 were defined as 'high'.

Statistical analyses. Correlation between the expression of Hsp105 and the clinicopathological characteristics of human bladder specimens were evaluated using the Pearson's χ^2 or the Fisher's exact tests. The CSS curves of patients with primary bladder cancer were determined using the Kaplan-Meier method and statistical significance was analyzed using the log-rank test. A multivariate analysis of the prognostic factors was performed using the Cox proportional hazards regression

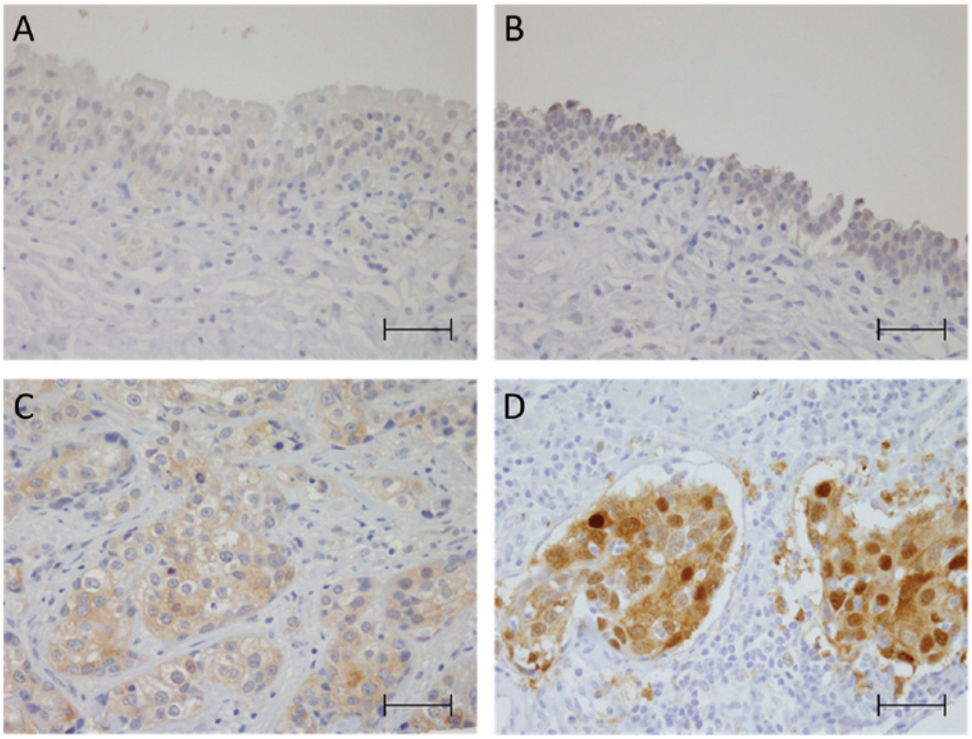
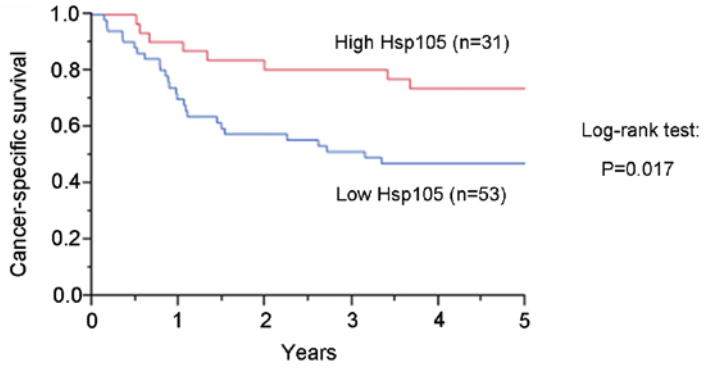


Figure 1. Expression of heat shock protein 105 (Hsp105) in (A and B) human non-cancerous bladder urothelium and (C and D) human primary bladder cancer by immunohistochemistry. Hsp105 expression (brown stain) with score (A) 0, (B) 1, (C) 2 and (D) 3. Scale bars, 50 μm.



Patients at risk

Hsp105	0-year	1-year	2-year	3-year	4-year	5-year
High	31	28	24	24	22	17
Low	53	34	28	25	21	19

Figure 2. Cancer-specific survival of 84 patients with primary bladder cancers according to heat shock protein 105 (Hsp105) scores. The P-value was calculated using the log-rank test.

model. JMP software (SAS Institute, Cary, NC, USA) was used for all the analyses. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of Hsp105 in human primary bladder cancer and correlation with clinicopathological characteristics. The immunoreactivity score for Hsp105 was low in all 4 non-cancerous bladder urothelium samples (score: 0 in 2 cases and 1 in 2 cases, Fig. 1A and B). In the 84 primary bladder cancer samples, the HSP score was low in 53 cases (63%, score 0 in 23 cases and 1 in 30 cases) and high in 31 cases (37%, score 2 in 20 cases and 3 in 11 cases, Fig. 1C and D).

Subsequently the correlation between Hsp105 scores and the clinicopathological characteristics of primary bladder cancer was investigated. As summarized in Table I, the Hsp105 score was not correlated with age, nuclear grade, or pathological tumor stage (P=0.575, 0.809 and 0.995, respectively). Female gender, lymphovascular invasion and lymph node metastasis exhibited a tendency towards lower Hsp105

Table II. Prognostic values of Hsp105 expression and clinicopathological characteristics for cancer-specific survival in 84 patients with primary bladder cancer.

Variable	Univariate analysis P-value	Multivariate Cox proportional hazards regression analysis		
		HR	95% CI	P-value
Hsp105 score (high vs. low)	0.017[a]	2.34	1.07-5.65	0.032[a]
Gender (male vs. female)	0.165	-	-	-
Age (<65 vs. >66 years)	0.470	-	-	-
Nuclear grade (G1/G2 vs. G3)	0.638	-	-	-
pT stage (pTa/pTis/pT1 vs. pT2/pT3/pT4)	<0.001[a]	6.69	1.76-44.0	0.003[a]
Lymph node metastasis (negative vs. positive)	<0.001[a]	1.62	0.746-3.55	0.220
Lymphovascular invasion (negative vs. positive)	<0.001[a]	1.40	0.548-3.93	0.492
Previous BCG therapy (yes vs. no)	0.099	-	-	-

[a]Statistically significant difference. HSP105, heat shock protein 105; HR, hazard ratio; CI, confidence interval; BCG, Bacillus Calmétte-Guerin.

scores, although the differences were not statistically significant (P=0.071, 0.061 and 0.175, respectively).

Correlation between expression of Hsp105 and the prognosis of patients with primary bladder cancer. The effect of the Hsp105 score on the CSS of patients with primary bladder cancer was investigated. The 5-year CSS for patients with high Hsp105 scores was 73.3% compared to 46.9% for those with low Hsp105 scores. Patients with high Hsp105 scores had a statistically better CSS compared to those with low Hsp105 scores (P=0.017; log-rank test, Fig. 2).

The prognostic value of Hsp105 expression and the clinicopathological characteristics for CSS was subsequently examined (Table II). In the univariate analysis, Hsp105 expression, pathological tumor stage, lymphovascular invasion and lymph node metastasis were variables significantly associated with survival. The multivariate Cox proportional hazard analysis demonstrated that Hsp105 expression was an independent indicator for CSS (P=0.032; hazard ratio=2.34), along with pathological tumor stage.

Discussion

Previous studies have reported that a high expression of Hsp105 in squamous cell carcinoma of the tongue (15) and lung adenocarcinoma (16) was associated with disease progression and/or poor prognosis. By contrast, the present study demonstrated that the high expression of Hsp105 was significantly associated with a favorable prognosis in urinary bladder cancer. These findings suggest that Hsp105 may play a different role in bladder cancer compared to squamous cell carcinoma of the tongue and lung adenocarcinoma. The poor prognosis observed in cases exhibiting a low expression of Hsp105 may be attributed to their inability to elicit an immune response.

In bladder cancer, the expression of other HSPs, such as Hsp27, -60, -70 and -90, was previously investigated. Lebret *et al* (18) reported that low expression of Hsp27 and Hsp60 were correlated with higher tumor stage, whereas low expression of Hsp60 and Hsp90 were correlated with infiltrating recurrence. Kamada *et al* (19) reported that Hsp27 knockdown inhibited tumor growth and enhanced sensitivity to chemotherapy in UMUC-3 human bladder cancer cells. Urushibara *et al* (20) reported that Hsp60 expression was associated with a good pathological response to neoadjuvant chemoradiotherapy. However, Syrigos *et al* (21) reported that Hsp70 expression was correlated with tumor grade, stage and overall survival. Those reports suggested that Hsp27, -60 and -90 may be involved in the suppression of bladder cancer, whereas Hsp70 may be involved in the progression of bladder cancer. Our results indicated that Hsp105 plays a protective role against bladder cancer progression, as do Hsp27, -60 and -90.

Notably, Hsp105 expression did not correlate with the pathological tumor stage, but correlated significantly with a favorable prognosis of bladder cancer patients. As tumors with lymphovascular invasion and lymph node metastasis exhibited a tendency for low Hsp105 scores, low Hsp105 expression may be associated with lymphovascular invasion and/or metastasis, rather than depth of tumor invasion, leading to the poor prognosis of patients. Furthermore, female gender was also associated with low Hsp105 scores. Generally, the female gender was shown to be associated with a higher recurrence rate and cancer-specific mortality following radical cystectomy (22,23), although the underlying causes have not been determined. A possible explanation is that the immunogenicity of Hsp105 may be associated with the inferior prognosis of female patients.

Zappassodi *et al* (17) reported that Hsp105-specific immune responses were induced by dendritic cell-based vaccination in relapsed B-cell non-Hodgkin lymphoma patients. The anti-lymphoma activity of the anti-Hsp105 antibody was demonstrated *in vivo* in xenotransplanted immunodeficient mice. Therefore, anti-Hsp105-specific immune responses may contribute to the prognosis of bladder cancer patients. However, in the present study, plasma or serum was not available from the patients whose specimens were used for TMA. Therefore, it remains to be elucidated whether anti-Hsp105 immune responses are induced in bladder cancer patients undergoing radical cystectomy. A prospective study is currently under planning to address this issue.

The multivariate analysis revealed that the high expression of Hsp105 was an independent factor for the prediction of a favorable prognosis of patients treated with radical cystectomy. Despite the advances in the methods of detection, surgical techniques, chemotherapy and irradiation, MIBC remains associated with a poor prognosis. The expression of Hsp105 may provide a novel prognostic marker in bladder cancer and enable the selection of a more appropriate treatment for patients with MIBC.

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