Original Article

Clinicopathological Significance of Serum NY-ESO-1 Antibodies in Patients with Gastric Cancer

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ABSTRACT

Background: Although clinicopathological significance of serum NY-ESO-1 antibodies has been evaluated in patients with esophageal cancer, little information was available in the patients with gastric cancer. We, therefore, evaluated pathological and prognostic significance of serum NY-ESO-1 antibodies (s-NY-ESO-1-Abs) in patients with gastric cancer.

Methods: Serum samples were obtained from 75 patients with gastric cancer before surgery. Serum samples were analyzed using an enzyme-linked immunosorbent assay system to detect s-NY-ESO-1-Abs. A cut-off optical density value was fixed at 0.165 (mean plus three standard deviations for serum samples from the healthy controls). Clinicopathological factors and patients prognosis were compared between seropositive and seronegative patients. Tissue microarray of surgically resected tumor specimens were evaluated for NY-ESO-1 immunoreactivities.

Results: The overall positive rate for s-NY-ESO-1-Abs was 8% (4.9% of stage I, 0% of stage II, 13% of stage III, and 38% of stage IV patients). s-NY-ESO-1-Abs was significantly associated with distant metastases ($P < 0.001$) and stage progression ($P = 0.005$). Although seropositive patients showed worse survival rates than seronegative patients, the difference was not statistically significant ($P = 0.106$). Based on the tissue microarray, the association between serum antibodies and immunoreactivities was not confirmed.

Conclusions: Although s-NY-ESO-1-Abs was significantly associated with distant metastasis and tumor progression, the prognostic impact of s-NY-ESO-1-Abs was not found to be statistically significant. The association between immunoreactivities and autoantibodies reaction was not confirmed in the current study using tissue microarray.

Toho J Med 3 (4): 101–106, 2017

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DOI: 10.14994/tohojmed.2017.3-4-101
Received Nov. 11, 2016: Accepted Aug. 16, 2017
Toho Journal of Medicine 3 (4), Dec. 1, 2017.
ISSN 2189–1990, CODEN: TJMOA2

KEYWORDS: NY-ESO-1, gastric cancer, serum autoantibody, tissue microarray

Introduction

NY-ESO-1 is a highly expressed tumor antigen in various solid tumors, particularly in esophageal cancer.[1] Recently, clinical trials of cancer vaccination were conducted using NY-ESO-1 as the target molecule.[2,3] Although clinicopathological significance of serum NY-ESO-1 antibodies (s-NY-ESO-1-Abs) was evaluated in the patients with esophageal cancer, little information was available in the patients with gastric cancer.[4–6] Moreover, there was little information about the association between presence of s-NY-ESO-1-Abs and immunoreactivities. We, therefore, evaluated clinicopathological and immunological significance of s-NY-ESO-1-Abs in patients with gastric cancer.

Materials and Methods

Collection of sera from patients and healthy controls

Serum samples were obtained before surgery from a total of 75 patients with gastric cancer who underwent radical surgery between 2011 and 2013 at Toho University Medical Center Omori Hospital. Written informed consent was obtained from all subjects. No preoperative therapy was performed. Patients included 47 men and 28 women with a median age of 69 years (range, 42-93 years). TNM stages of each tumor were classified on the basis of general rules for the clinical and pathologic study of gastric cancer (TNM Classification of Malignant Tumours, 7th Edition) as follows: 41 were classified as stage I, 18 as stage II, eight as stage III, and eight as stage IV. Serum samples were also obtained from 74 healthy volunteers. Each serum sample was centrifuged at 3,000× g for 5 min, and the supernatant was stored at −80℃ until the assay. Repeated thawing and freezing of the samples was avoided. The present study was approved by the Institutional Review Board of Toho University School of Medicine (#22-112, #22-047).

Tissue microarray construction and pathologic analyses

A tissue microarray was constructed using a total of 75 gastric cancer specimens from formalin-fixed, paraffin-embedded archived tissue samples of the Toho University Medical Center Omori Hospital. The use of tissue microarrays for research purposes had been approved by the local ethics committee. For tissue microarray construction, only surgical specimens of previously untreated patients were used. Consecutive freshly cut sections of tissue microarrays were used for immunohistochemical analysis and hemotoxylin and eosin stained reference. Tissue cylinders with a diameter of 5 mm were punched from tumor areas of gastric cancer tissue block and normal mucosa paraffin blocks using a custom-made precision instrument. Tissue blocks of one or two cancer tissues and one adjacent normal tissue were cored using a tissue microarrayer type KIN (Azumaya, Tokyo, Japan). All cases included were reviewed by two pathologists. The pathologic stage was obtained from the primary report of the Department of Clinical Pathology. Raw survival data were available from all patients and the mean follow-up period was 41 months (range, 4-55 months).

Immunohistochemistry

Immunohistochemical analyses were performed on 4-μm thick tissue microarray sections. Immunoperoxidase staining for NY-ESO-1 was performed using the Ventana Benchmark XT (Ventana Medical Systems*1, Tucson, AZ, USA) automated slide-staining system. Sections were deparaffinized and pretreated with Cell Conditioning 1 (CC1, Ventana Medicals Systems*1) using mouse monoclonal antibody (clone E978, Sigma-Aldrich, 3050 Spruce Street, St, Louis, MO, USA). The primary antibody reaction was performed for 32 min at room temperature, visualized using Ventana's DAB detection kits and iView DAB detection kit (Ventana Medical Systems*1), and counter-stained with Hematoxylin II (Ventana Medical Systems*1) and Bluing Reagent (Ventana Medical Systems*1). NY-ESO-1 antibody was diluted to 1/60 and used with the iVIEW DAB detection kit (Ventana Medical Systems) and an Endogenous Biotin blocking kit (Ventana Medical Systems). As a positive control of NY-ESO-1 immunostaining, human testis tissues were used. In addition, negative controls were prepared by adding REAL Antibody Diluent (Dako, Agilent Technologies Company, Santa Clara, CA, USA) instead of the primary antibody. We considered cells even partially displaying a positive result for NY-ESO-1 by immunohistochemical staining using tissue microarrays as a positive result. Tumors that displayed staining with an intensity of 2 or 3+ (moderate or strong) and presenting >2% of the tissue cells staining for the antigen were considered positive.[7]

Table 1 Relationships among s-NY-ESO-1-Abs and clinicopathological factors.

	Serum NY-ESO-1 antibodies		
	negative	positive	P value *
Age			
≥65	25 (33%)	1 (1.3%)	0.334
>65	44 (59%)	5 (6.7%)	
Gender			
Male	4	4 (5.3%)	0.833
Female	26 (35%)	2 (2.7%)	
Histological type			
Differentiated	32 (43%)	3 (4.0%)	0.865
Undifferentiated	37 (49%)	3 (4.0%)	
Depth of tumor invasion			
pT1-T2	40 (53%)	2 (2.7%)	0.244
pT3-T4	29 (39%)	4 (5.3%)	
Lymph node metastasis			
negative	46 (61%)	2 (2.7%)	0.101
positive	23 (31%)	4 (5.3%)	
Distant metastasis			
negative	64 (85%)	3 (4.0%)	<0.001
positive	5 (6.7%)	3 (4.0%)	
Stage			
I-II	57 (76%)	2 (3.7%)	0.005
III-IV	12 (16%)	4 (5.3%)	

*Fisher's exact probability test.

Enzyme-linked immunosorbent assay

Sera from a total of 75 patients and 74 healthy controls were analyzed with enzyme-linked immunosorbent assay, and levels of serum s-NY-ESO-1-Abs were assessed using optical density values as previously described.[6] The levels of s-NY-ESO-1-Abs were divided into two groups: normal optical densities that were below the cutoff level of 0.165 (calculated as the mean plus three standard deviations of the values in healthy donors) and positive values that were higher than 0.165. All healthy controls were negative for s-NY-ESO-1-Abs.

Statistical analysis

Fisher's exact test was used to assess the associations between s-NY-ESO-1-Abs, NY-ESO-1 immunostaining, and clinicopathological variables. Overall survival rate was calculated using the Kaplan-Meier product limit estimate. Survival differences between groups were determined using the log-rank test. Mann-Whitney U test was used to compare optical density values between two groups. A p-value of <0.05 was defined as a statistically significant difference.

Results

Relationship among s-NY-ESO-1-Abs and clinicopathological factors

The overall positive rate for s-NY-ESO-1-Abs was 8% (4.9% of stage I, 0% of stage II, 13% of stage III, and 38% of stage IV patients). Presence of s-NY-ESO-1-Abs was significantly associated with distant metastasis ($P<0.001$) and tumor stages ($P=0.005$). The positive rate of s-NY-ESO-1-Abs was higher in lymph node-positive patients than lymph node-negative patients, however the difference was not statistically significant ($P=0.101$). Other clinicopathological factors were not associated with the presence of s-NY-ESO-1-Abs (Table 1).

Prognostic significance of s-NY-ESO-1-Abs

Although the difference was not statistically significant, seropositive patients showed poorer survival than seronegative patients (Fig. 1, P=0.106).

NY-ESO-1 immunoreactivity

The overall positive rate of NY-ESO-1 staining was 11% (8 of 75). Two representative immunoreactivities of tissue microarrays were shown in Fig. 2. The positive rate for immunoreactivity of NY-ESO-1 was 11% (9.8% of stage I,

28% of stage II, 25% of stage III, and 0% of stage IV patients).

Presence of s-NY-ESO-1-Abs and NY-ESO-1 staining

The correlation between the presence of s-NY-ESO-1-Abs and NY-ESO-1 staining was determined in gastric cancer tissues (Table 2). Only one of eight (13%) patients with positive staining was revealed to be seropositive. On the other hand, five of 67 (7%) patients with negative staining were revealed to be seropositive. There was no significant association between NY-ESO-1 staining and antibody reactions (P = 0.620). Although optical density values (mean ± standard deviation) of s-NY-ESO-1 were slightly higher in the patients with positive staining than the patients with negative staining (0.036 ± 0.102 vs 0.031 ± 0.052, P = 0.194), the difference was not statistically significant (Fig. 3).

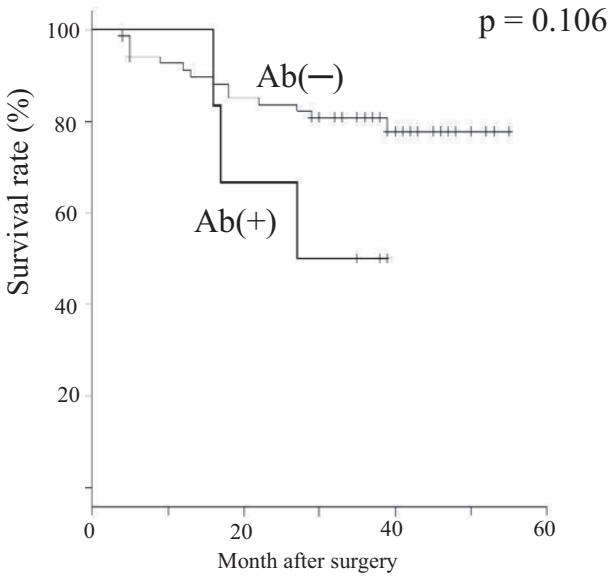

Fig. 1 Overall survival curves according to the status of serum NY-ESO-1 antibodies.

Discussion

Fujiwara et al. reported that the positive rates of s-NY-ESO-1-Abs in patients with early and advanced gastric cancer were 7.2% and 16.8%, respectively.[4] The overall positive rate of our present series was relatively lower than that of Fujiwara et al. This difference was partly explained by the higher ratio of early stage of gastric cancer in our study subjects. The positive rate of the NY-ESO-1 staining in surgically resected specimens of gastric cancer was reported as 30%.[3] Serum antibody induction was generally reported to be significantly associated with tumor antigen expression.[8, 9] However, our results could not confirm such associations. A previous report also noted that there was no statistically significant association between s-NY-ESO-1-Abs and NY-ESO-1 staining.[10] This discrepancy was partly explained by the heterogenic expression pat-

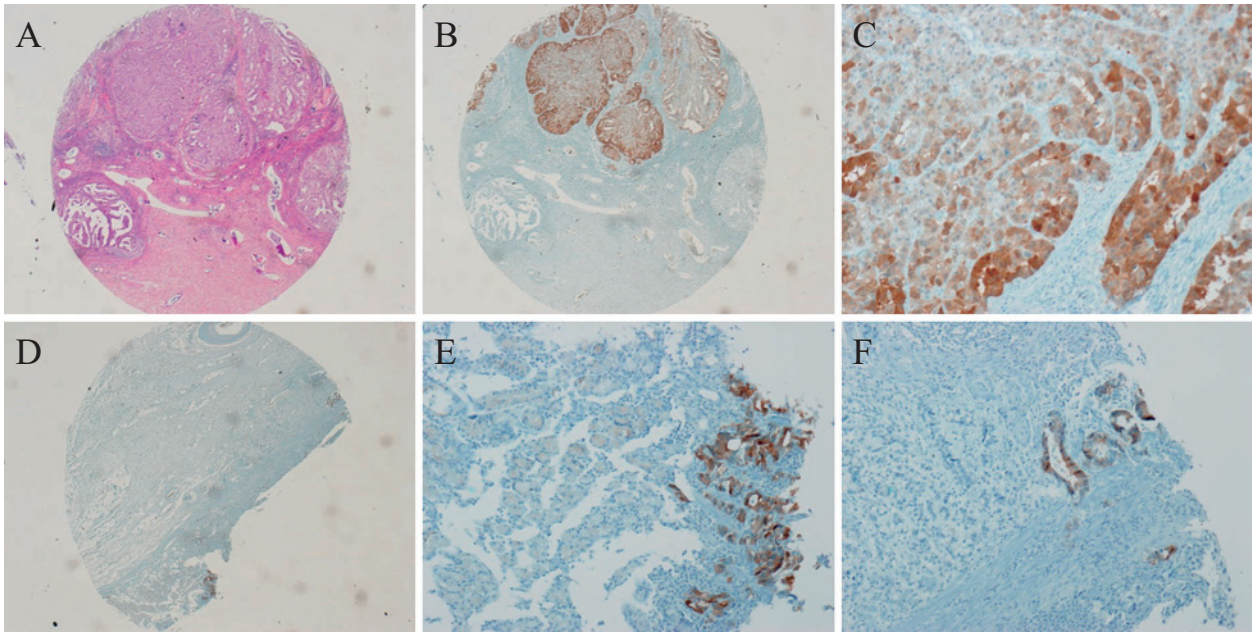

Fig. 2 Histological findings of the tissue microarray. Hematoxylin and eosin stain (A), the case in which more than 50% of cells showed positive immunoreactivity for NY-ESO-1 (B and C), the case in which more than 2% of cells showed positive immunoreactivity for NY-ESO-1 (D, E, and F).

Table 2 Relationship between the presence of serum anti-NY-ESO-1 antibody and NY-ESO-1 staining in gastric cancer tissues.

	Serum anti-NY-ESO-1 antibodies		
	positive (n = 6)	Negative (n = 69)	p value *
NY-ESO-1 immunostaining			
Positive (n = 8)	1 (13%)	7 (87%)	p = 0.620
Negative (n = 67)	5 (7%)	62 (93%)	

*Fisher's exact probability test.

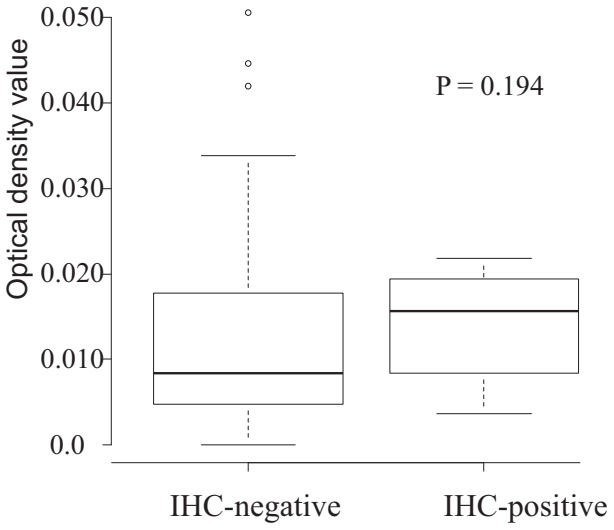

Fig. 3 Optical density values for s-NY-ESO-1-Abs titers in NY-ESO-1 staining negative group and NY-ESO-1 staining positive group.

tern of NY-ESO-1 in cancer tissues; this heterogenic expression pattern was similar to that of HER2. Because of the heterogeneous expression of HER2 in gastric cancer tissues, at least five or more biopsy samples were required to be evaluated for HER2 expression.[11] Such heterogeneity might also present in NY-ESO-1 antigen expression in gastric cancer tissues. Although the diameter of tissue microarray used in this present study was much larger than endoscopic biopsy specimens, heterogeneous expression still affected the association between s-NY-ESO-1-Abs and NY-ESO-1 staining. Since s-NY-ESO-1-Abs was found to be associated with TNM factors, s-NY-ESO-1-Abs could be used as a new biomarker to predict the TNM stage and/or patient survival. Because of the small sample size of the present study, the differences between survival curves of seropositive and seronegative patients might not have reached a statistically significant level. Further studies with larger sample size are required to obtain a final conclusion for the clinicopathological significance of s-NY-ESO-1-Abs.

Acknowledgements

We thank the Ministry of Education, Culture, Sports, Science, and Technology of Japan for Grants-in-Aid for Scientific Research (nos. 23591948 and 15K10117). We also thank Ms. Seiko Otsuka for sample collection.

Conflicts of interest: Hideaki Shimada received a research grant from Medical & Biological Laboratories Co., Ltd., Nagoya, Japan. Akiko Kuwajima is an employee of the Medical & Biological Laboratories Co., Ltd., Nagoya, Japan. The other authors have no conflicts of interest.

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