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Analysis of the Survival Period in Resectable Stage IV Gastric Cancer

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Background: Patients with stage IV gastric cancer usually have a poor prognosis, but some patients with resectable cancer survive for more than 5 years. We aimed to study the correlation of protein expression and survival in resectable stage IV gastric cancer.

Patients and Methods: Tissue samples of 42 patients with resectable stage IV gastric cancer were stained immunohistochemically for the mutant p53 protein and heat shock protein-27 (hsp27). The correlation between protein expression and clinicopathological factors was investigated. Furthermore, prognostic value of each factor was analyzed.

Results: Univariate analysis showed that pN factors (Japanese classification, $P = .028$; International Union Against Cancer classification, $P = .024$), blood vessel invasion ($P = .043$), hsp27 overexpression ($P = .019$), and the index of p53 and hsp27 overexpression ($P = .0026$) had a prognostic influence. Only Lauren classification, however, revealed the prognostic influence in multivariate analysis ($P = .046$).

Conclusions: These results suggest that immunostaining of tumor specimens for p53 and hsp27 and clinicopathological analysis may help predict the survival of patients with resectable stage IV gastric cancer.

Key Words: hsp27—p53—Resectable stage IV gastric cancer—Lymph node metastasis.

Gastric carcinoma is one of the most common cancers in Japan. Early detection improves the prognosis. In general, patients with stage IV gastric cancer have a poor prognosis despite the development of surgical treatment and anticancer chemotherapy. Furthermore, not all cases of stage IV gastric cancer are resectable. Interestingly, some patients have a longer survival after surgery.[1,2] Adjuvant chemotherapy usually is required after surgery in patients with advanced gastric carcinoma, but responses to chemotherapy vary.

The tumor suppressor p53 has been related to the carcinogenesis or progression of gastric cancer. The mutant p53 protein was reported to cause higher risk of lymph node metastasis[3–6] and anticancer drug resistance by inhibiting apoptosis.[7] Furthermore, mutation of the p53 gene has been linked to a poor prognosis of gastric carcinoma.[3,4,6–10] Heat shock protein-27 (hsp27), a stress protein, also has been responsible for the resistance to adjuvant chemotherapy. Overexpression of hsp27 led to a poor prognosis of ovarian[11,12] and breast cancer.[13] In addition, hsp27 was shown to suppress cytotoxicity of tumor necrosis factor-α[14,15] and inhibit apoptosis.[16,17]

Lymph node metastasis is an important prognostic factor in advanced gastric cancer.[1,18] The Japanese Classification of Gastric Carcinoma determines pN category based on the location of metastatic lymph nodes,[19] whereas the International Union Against Cancer (UICC) classification determines pN category based on the number of metastasis positive lymph nodes.[20] It is not clear which classification system is more effective in predicting the prognosis of progressive gastric cancer.

In the present study, we used immunohistochemistry to investigate the relationship between expression of p53 or hsp27 and clinicopathological factors and to predict the prognosis of stage IV gastric carcinoma. We also compared the pN factors of the Japanese and the UICC classification in stage IV gastric cancer.

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PATIENTS AND METHODS

Patients

Forty-two patients with stage IV gastric carcinoma (26 males and 16 females, ranging in age from 42 to 85 years, mean age 66.2 years) were included in the present study. These patients had surgical treatment that included gastric resection and extended lymph node dissection at the Department of Surgery II of the Oita Medical University from 1991 to 1996. Patients without macroscopic residual cancer except distant metastasis were included from this study.

Patients were classified with the Japanese system by the Japanese Research Society for Gastric Cancer.[19] The pM category included liver metastasis, peritoneal dissemination, and other distant metastasis. The pN factors of the Japanese classification system were compared with those of the tumor, node, metastasis classification system by UICC.[20] By contrast, other factors, the pT and pM categories, showed almost the same classification in both Japanese and VICC classifications. pN factors of the two classification systems were divided into two groups (pN = 0, 1; pN = 2, 3). Similarly, lymphatic (ly) and blood vessel (v) invasion was divided into two groups (0, 1; and 2, 3). pN1 or ly1 and v1 were regarded as negative cases because only stage IV gastric cancer patients who have progressive invasion and lymph node metastasis or vessel invasion were investigated in the present study.

Furthermore, these patients were classified into two groups according to the Lauren classification histopathologically.[21] Not only their morphologic characteristics but also mucin stainings (periodic-acid Schiff, Alcian blue/diastase) were used for the assessment, and their predominant characteristics were accepted for histopathological classification.

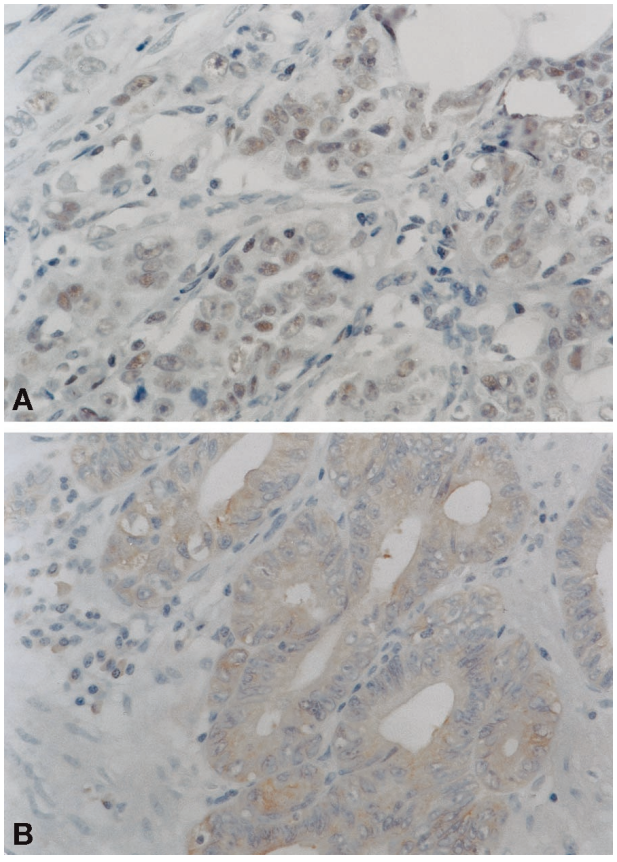

FIG. 1. Immunohistochemical staining for p53 and hsp27 proteins. (A) p53: the nuclei of cancer cells were stained brown. (B) hsp27: cytoplasm of cancer cells were stained brown.

Chemotherapy

After surgery, patients entered our chemotherapy protocol. The therapy consisted of four cycles per month. They received 5-fluorouracil (250 mg) and cisplatin (5 mg) daily by continuous intravenous drip for 5 days except Saturday and Sunday in each cycle. The adjuvant therapeutic indication was based on the condition of the patient, such as heart, renal, or hepatic functions. Patients were followed up every 3 months in our hospital. Patients with bone marrow suppression during chemotherapy were excluded from the study.

Immunohistochemistry

All resected specimens were fixed in 10% buffered formalin for 24 hours and embedded in paraffin. Sections (4 μm) were placed on silane-coated slides. After deparaffinization and rehydration, they were put into 3% hydrogen peroxide for 20 minutes to inactivate endogenous peroxidase. They were then autoclaved at 121°C in citrate buffer (10 mM, pH 6.0) for 10 minutes for antigen activation. After cooling at room temperature for 30 minutes, the specimens were incubated further with normal rabbit serum for 15 minutes at room temperature. They were incubated with either anti-p53 monoclonal antibody (DO-7; 1:50; Dako, Carpinteria, CA) or anti-hsp27 monoclonal antibody (1:30; Novocastra, Newcastle upon Tyne, UK) for 16 hours at 4°C. Immunohistochemical staining was performed by using a standard avidin-biotin-peroxidase complex technique with a streptavidin-biotin-peroxidase kit (Nichirei, Tokyo, Japan). We used 3,3'-diaminobenzidine as the chromogen. The counterstaining of nuclei was performed with hematoxylin.

As positive control, breast cancer that revealed positive reactions for p53 and hsp27 was used. Negative control was performed without primary antibodies.

Immunostaining for p53 protein was measured by the number of cancer cells with stained nuclei: 0% to 10% (−), 10% to 50% (+), and >50% (++). Immunostaining for hsp27 protein was measured by the area of immunopositive cells: without expression (−), patchy expression (+), and widespread expression (++). We divided these cases into three groups because the tumor was large and heterogeneous. The index calculated by the total of the results of immunohistochemical staining for p53 and hsp27 (negative was evaluated as 0, + as 1, and ++ as 2) also was examined.

To evaluate immunostaining, two physicians (S.T. and T.S.) observed independently without knowledge of the patients' data. Forty-one cases (97.6%) for p53 and 40 cases (95.2%) for hsp27 received the same evaluation. The remaining cases were reevaluated, and agreement was obtained.

Statistical Methods

The Mann-Whitney *U*-test was used to analyze the correlation between protein expression and clinicopathological factors. The Kaplan-Meier method was used to measure the prognostic influences from the month after surgery. The differences were examined by using the log-rank test. Cox proportional hazard model was used in the multivariate analysis. A level of $P < .05$ was considered statistically significant.

RESULTS

Immunostaining for p53 and hsp27

Figure 1 shows the staining pattern of p53 and hsp27. We detected p53 expression only in the nuclei (Fig. 1A), and hsp27 was detected in the cytoplasm (Fig. 1B). Tumors from 21 of 42 patients (50%) stained positively with the anti-p53 antibody. Among them, 5 of 42 (12%) were + and 16 of 42 (38.1%) were ++. Positive for hsp27 was detected in 22 of 42 patients (52.4%). Among them, 11 of 42 (26.2%) were + and another 11 of 42 (26.2%) were ++.

Correlation Between Expression of p53 or hsp27 and the Clinicopathological Factors

The result of p53 immunostaining correlated significantly with only the pN category of the Japanese system ($P = .038$) but not with the UICC system (Table 1). On

TABLE 1. *Correlations betwwen p53 overexpression and clinicopathological factors*

	p53 (−)	p53 (+)	p53 (++)	*P* value
Age				
<65	8	3	7	
>65	13	2	9	.68
Sex				
Male	13	3	10	
Female	8	2	6	.98
Configuration				
Scirrhous	5	2	6	
Nonscirrhous	16	3	10	.36
Lauren classification				
Intestinal type	8	2	6	
Diffuse type	13	3	10	.98
pT				
Non-pT4	10	1	4	
pT4	11	4	12	.14
pN (Japanese classification)				
pN = 0,1	10	3	2	
pN = 2,3	11	2	14	.038
pN (UICC classification)				
pN = 0, 1	7	1	4	
pN = 2,3	14	4	12	.38
pM				
Negative	8	4	1	
Positive	13	1	10	.91
Lymphatic invasion				
ly = 0,1	7	1	6	
ly = 2,3	14	4	10	.84
Blood vessel invasion				
v = 0,1	17	5	12	
v = 2,3	4	0	4	.72

Values determined by Mann-Whitney's *U*-test. p53, mutant protein; (−), 0% to 10% cancer cells with stained nuclei; (+), 10% to 50%; (++), >50%; UICC, International Union Against Cancer.

the other hand, hsp27 immunostaining did not correlate with either of the classification systems (Table 2).

Statistical Analysis Among Immunohistochemical, Clinicopathological Factors, and Survival Period

Univariate analysis showed that the survival period after surgery correlated significantly with the pN categories of both the Japanese ($P = .028$, Fig. 2A) and the UICC systems ($P = .024$), blood vessel invasion ($P = .043$), hsp27 expression ($P = .019$, Fig. 2C), and the index of p53 and hsp27 ($P = .0026$, Fig. 2D). However, p53 positive was not a prognostic factor for survival period (Fig. 2B). In addition, surgical curability (R0 or not) was not found to be a prognostic factor in the present study. In the multivariate analysis, only Lauren classification but not other factors proved to be a statistically relevant prognostic factor for survival period of patients ($P = .046$, Table 3).

Correlation Between Protein Expression and Survival

We also examined the patients with longer survival who lived more than 3 years (36 months) after surgery. The results are summarized in Table 4. Only six patients were studied in this group (three males and three females, mean age 68.7 years). Among these patients, three had intestinal-type gastric cancers and the other three revealed diffuse type tumors. One patient showed scirrhous cancer type. Five patients showed tumor invasion to adjacent organs. Severe lymph node metastasis was observed in one patient according to the Japanese system, whereas two patients had lymph node metastasis according to the UICC system. Only one patient showed distant metastasis. None of the patients had severe blood vessel invasion, but five of the patients had severe lymphatic invasion (ly2 and 3). Good prognosis was observed in four of six patients who had undergone R0 curative surgery. Interestingly, five of the six patients showed immunonegative for p53 and hsp27 expression. The remaining patient was also negative for p53 but was positive for hsp27.

DISCUSSION

Some longer survival patients with resectable stage IV gastric cancer have been reported.[1,2] Lymph node metas-

TABLE 2. *Correlations between hsp27 overexpression and clinicopathological factors*

	hsp27 (−)	hsp27 (+)	hsp27 (++)	*P* value
Age				
<65	8	5	5	
>65	12	6	6	.74
Sex				
Male	11	9	6	
Female	9	2	5	.73
Configuration				
Scirrhous	6	3	4	
Nonscirrhous	14	8	7	.78
Lauren classification				
Intestinal type	7	5	4	
Diffuse type	13	6	7	.83
pT				
Non-pT4	9	3	3	
pT4	11	8	8	.27
pN (Japanese classification)				
pN = 0,1	9	5	1	
pN = 2,3	11	6	10	.084
pN (UICC classification)				
pN = 0,1	6	5	1	
pN = 2,3	14	6	10	.49
pM				
Negative	8	5	5	
Positive	12	6	6	.74
Lymphatic invasion				
1y = 0,1	7	5	2	
1y = 2,3	13	6	9	.5
Blood vessel invasion				
v = 0,1	18	9	7	
v = 2,3	2	2	4	.09

Values determined by Mann-Whitney *U*-test. hsp. heat shock protein; (−), without expression of immunopositive cells; (+), patchy expression; (++), widespread expression; UICC, International Union Against Cancer.

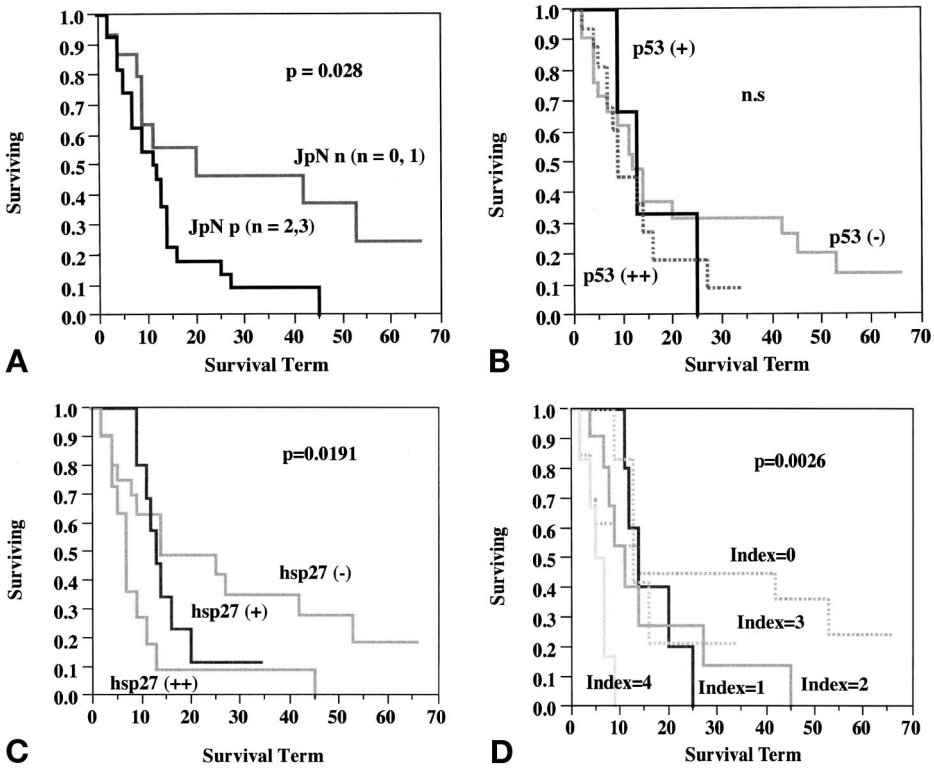

Fig. 2. (A) Survival curve of resectable stage IV gastric patients stratified by pN factor of the Japanese classification (JpN; $P = .028$); n, n = 0, 1; p, n = 2 and 3. (B) Survival curve of resectable stage IV gastric patients stratified by p53 overexpression (no significant difference, ns). (C) Survival curve of resectable stage IV gastric patients stratified by hsp27 overexpression ($P = .019$). (D) Survival curve of resectable stage IV gastric patients stratified by the index of p53 and hsp27 overexpression ($P = .0026$).

tasis is known to be one of the most important prognostic factors of advanced gastric cancer.[18] Therefore, R0 surgery together with extended lymphadenectomy is required to improve the prognosis of these patients.[1,2]

In the univariate analysis, pN factors of both the Japanese and the UICC systems and blood vessel invasion correlated with the survival of patients who had the cancer. In addition, pN factors of both systems revealed prognostic significance at almost the same level. Determination of pN staging of stage IV gastric cancer in the UICC system is based on the number of metastasis-positive lymph nodes, whereas that of the Japanese system is based on the area of lymph node metastasis. In fact, both systems reflect the prognosis well, although they were not the independent prognostic factors. However, compared with other reports, no clinicopathological factors had an independent prognostic power in multivariate analysis in the present study.[1,2,18] Although, to the

TABLE 3. *Multivariate Cox proportional hazards analysis of stage IV gastric cancer*

Covariate	Category	Risk ratios	*P* value
Sex	Male	0.51	.27
Age	<65	0.59	.29
Configuration	Scirrhous	0.37	.11
Invasion to adjacent organ	Invaded	0.41	.17
Japanese pN	Positive	2.86	.17
UICC pN	Positive	1.58	.48
Distant metastasis	Positive	2.46	.27
Lauren classification	Intestinal	0.31	.046
Lymphatic invasion	Positive	1.56	.41
Blood vessel invasion	Positive	2.11	.36
Surgical curability	Non-R0	1.02	.98
p53	++	5.7	.21
hsp27	++	8.27	.22
Index of p53 and hsp27	Index 4	1	.19

UICC, International Union Against Cancer; hsp, heat shock protein; ++ (for p53), >50% cancer cells with stained nuclei; ++ (for hsp27), widespread expression of immunopositive cells.

TABLE 4. *Long survival cases of resectable stage IV gastric cancer*

No.	Sex	Age	Conf	Lauren	pT4	Sev	pN (U)	pM	Sev ly	Sev v	Surgery	p53	hsp27
1	Female	84	Non-sci	Int	Pos	Neg	Pos	Neg	Pos	Neg	Non-R0	(−)	(−)
2	Male	61	Non-sci	Int	Neg	Pos	Pos	Neg	Pos	Neg	R0	(−)	(++)
3	Male	66	Non-sci	Dif	Pos	Neg	Neg	Neg	Pos	Neg	R0	(−)	(−)
4	Male	73	Non-sci	Int	Pos	Neg	Neg	Neg	Pos	Neg	R0	(−)	(−)
5	Female	63	Sci	Dif	Pos	Neg	Neg	Pos	Pos	Neg	Non-R0	(−)	(−)
6	Female	65	Non-sci	Dif	Pos	Neg	Neg	Neg	Neg	Neg	R0	(−)	(−)

Conf, configuration; sci, scirrhous; neg, negative; pos, positive; sev, severe; (J), Japanese classification; (U), Interational Union Against Cancer classification; int, intestinal type; dif, diffuse type; ly, lymphatic invasion; v, blood vessel invasion; hsp, heat shock protein; (−) (for p53), 0% to 10% cancer cells with stained nuclei; (−) (for hsp27), without expression of immunopositive cells; (++) widespread & expression of immunopositive cells.

surgeon's regret, R0 surgery was not a significant prognostic factor for long-term survival in the present study, radical gastrectomy with extended lymph node dissection should be required in the surgical treatment of stage IV gastric cancer because both pN factors revealed a significant prognostic power in the univariate analysis.

It is of interest that only the Lauren classification had an independent prognostic power in this series. The result of the present study did not agree with Lauren's results,[21] perhaps because only "resectable" stage IV cases were used in the present study against Lauren's overall analysis.

The tumor suppressor p53 plays an important role in the control of cell cycle and apoptosis. Clinical studies have linked either a p53 gene mutation or p53 overexpression to aggressive tumor behavior and a poor prognosis in gastric cancer.[3,4,6,8–10] Authors have reported p53 overexpression to be a predictor of poor response to 5-fluorouracil and cisplatin chemotherapy in gastric cancer patients.[7] Nevertheless, others have reported that p53 was not an independent prognostic factor by multivariate analysis.[22–25] Although our data showed that p53-negative cases tended to have a longer survival period, p53 status did not influence the prognosis significantly. These results suggest that overexpression of p53 protein is associated with cancer progression before advancing to stage IV. However, p53 mutation detected by single-strand conformation polymorphism analysis, rather than p53 protein accumulation detected immunohistochemically, was an independent prognostic factor.[3] Further study of p53 mutation is required.

The hsp27, a stress protein, has been implicated with anticancer drug resistance.[11,13,26] Anticancer drug resistance was considered to cause poor prognosis in human tumors. Overexpression of hsp27 and a negative prognosis of ovarian and breast carcinoma have been linked.[11–13] Doxorubicin resistance, for instance, is a consequence of hsp27 expression in breast cancer patients.[27] The protein also protects the cells from cytotoxic effect of cisplatin.[28] Because the supplemental chemotherapy that used 5-fluorourasil and cisplatin after surgery was performed in our institute, the results of this study agree with previous reports. However, the correlation between hsp27 overexpression and the prognosis of progressive gastric cancer patients has not been clear until now. To our knowledge, this is the first report to clarify this correlation. Furthermore, hsp27 has been shown to inhibit tumor necrosis factor-α-induced cytotoxicity,[14,15,26] and it plays an important role in apoptosis suppression.[16,17] Clearly, hsp27 is a negative prognostic factor in cancer patients. Although hsp27 overexpression revealed poor prognosis of stage IV gastric cancer patients in the univariate analysis, it was not an independent prognostic factor in multivariate analysis. Likewise, the index of p53 and hsp27 was a negative prognostic factor in univariate analysis but not in multivariate analysis.

However, interestingly, five of six patients with longer survival revealed a similar pattern of protein expression. They were immunonegative for both p53 and hsp27. Therefore, we believe that immunohistochemical analysis is useful in predicting the prognosis of stage IV gastric cancer patients.

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