J Korean Surg Soc 2011;80:172-181
DOI: 10.4174/jkss.2011.80.3.172

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Expression of c-*erb*B2 and *p53* in Curatively Resected Gastric Cancer: Correlation with Clinicopathologic Features and Prognosis

Department of Surgery, College of Medicine, The Catholic University of Korea, Seoul, Korea

Hoon Hur, M.D., **Jeong Min Yi,** M.D., **In Kyu Lee,** M.D., Ph.D., **Kyo Young Song,** M.D., **Wook Kim,** M.D., Ph.D., **Cho Hyun Park,** M.D., Ph.D., **Hae Myung Jeon,** M.D., Ph.D.

Purpose: We investigated the correlation between expression of c-erbB-2 and *p53* proteins and clinicopathologic features of gastric cancer to reveal prognostic factors.
Methods: 125 patient records under going curative resection for gastric carcinoma at our institution from January 2000 to June 2003 were collected. Surgical specimens embedded in paraffin block were evaluated for *p53* and c-erbB-2 protein as detected by immunohistochemistry.
Results: Samples from 30 cases (24.0%) of 125 patients with gastric adenocarcinomas demonstrated positive staining for c-erbB-2 and 72 patients (57.6%) showed positive nuclear staining for *p53* protein. c-erbB-2 stained tumors were significantly associated with the depth of tumor invasion (P=0.022), lymph node metastasis (P=0.004) and lymphatic invasion (P=0.019). In a subgroup of patients with gastric carcinoma not exposed to serosa (n=91), expression of both c-erbB-2 and *p53* significantly related with poor disease-free survival (OR=5.107) and survival (OR=4.449) in multivariate analysis.
Conclusion: When patients with gastric adenocarcinoma showed expression of c-erbB-2 with *p53*, they were associated with aggressive pathologic features. In the subgroup of patients with gastric adenocarcinoma that did not involve serosa, expression of c-erbB-2 combined with *p53* could become a predictive factor for recurrence and survival in multivariate analysis. **(J Korean Surg Soc 2011;80:172-181)**

Key Words: Gastric adenocarcinoma, *p53*, c-erbB-2, Prognosis

INTRODUCTION

Gastric cancer is one of the most common malignancies worldwide. It remains fatal in spite of early diagnosis and the development of chemotherapeutic agents to combat it.(*1*) The most important cause of death after curative treatment for locally advancced gastric cancer is recurrence of primary tumor. The recurrence may be ameliorated by appropriate adjuvant treatment using agents targeting specific molecules. The mechanism of new drug for target treatment would involve clarifying the mechanism of action of various genes associated with the carcinogenesis of gastric cancer. Before attempting to clarify the mechanism, confirming whether tumor expressions of specific oncogenes or tumor suppressor genes are associated with the clinicopathologic features of gastric cancer as well as the prognosis of patients, is important.

The c-erbB-2 gene is a proto-oncogene located on chromosome 17. It expresses HER2/*neu* protein, one of the epithelial growth factor receptor (EGFR) families, and has tyrosine kinase (TK) activity, which mediates cancer proliferation.(*2*) The *p53* gene is also located on chromosome 17. It is a representative tumor suppressor gene, and mutations of this gene are found out in most tumors originating from the gastrointestinal system, urogenital system, and skin.(*3*) The wild-type *p53* gene is involved in

Correspondence to: Hae Myung Jeon, Department of Surgery, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 505, Banpo 4-dong, Seocho-gu, Seoul 137-701, Korea. Tel: 02-2258-2683, Fax: 02-595-2992, E-mail: hmjeon@catholic.ac.kr
Received February 6, 2010, Accepted May 10, 2010

the differentiation, proliferation and apoptosis of cells, whereas the mutant type is considered to be the cause of atypical cell growth.(*4*)

However, the correlation of expression of c-*erb*B-2 and *p53* with clinicopathologic and prognostic features is controversial.(*5-10*) We investigated if there is a correlation between abnormal expression of c-*erb*B-2 and *p53* genes and the clinicopathologic features of the tumor. In addition, it was studied that the expression of two gene can be prognostic factors of gastric adenocarcinomas.

METHODS

1) Patients and tissues

One hundred twenty-five patients who underwent curative gastric resection for gastric adenocarcinoma from January 2000 to June 2003 in St. Mary's Hospital were enrolled for this study. In this period, cancer tissues from all patients were investigated the expression of c-erbB-2 and *p53*. The results of staining were reported accompanying with pathologic results. We retrospectively reviewed this data. We obtained informed consent from all patients provided about supplement of tissues and immunohistochemical staining.

Curative gastric resection was performed according to treatment guideline for gastric cancer suggested by Japanese Gastric Cancer Association. After curative operation, adjuvant chemotherapy based on 5-FU was performed for patients diagnosed with over stage II according to 6th edition of American Joint Committee on Cancer (AJCC) staging system.

Cancer stage according to 6th edition of AJCC satging, tumor invasiveness, involvement of lymph nodes, histological features and the Laurén classification were evaluated by reviewing pathology reports. Recurrence and death were determined according to the follow-up database at our institution.

2) Immunohistochemical staining

Tumor tissues were fixed in 10% formalin and embedded in paraffin. Immunohistochemical staining was carried out using anti-HER-2/neu (Dako, Glostrup, Denmark) as the primary antibody for c-erbB-2. After making slices using a microtome, tissue sections (4 μm) were immersed in xylene solution to remove residual paraffin and hydrated in an alcohol series. Sections were boiled for 5 min to retrieve antigenicity in citrate buffer (pH 6.0) and left for 30 min at room temperature.

After exhausting endogenous peroxidase for 10 min with H_2O_2 in methyl alcohol, sections were washed thrice with phosphate-buffered saline (PBS). Sections were blocked for 30 min with blocking solution (Histostain^{TM} kit, Zymed Company, San Francisco, CA, USA) at room temperature. Sections were then incubated with anti-HER-2/neu (1 : 200, Dako) at room temperature. After rinsing thrice with PBS, sections were incubated with biotinylated anti-mouse IgG (1 : 300; Zymed). After washing, sections were incubated with avidin-alkaline phosphatase for 7 min at 40°C. Sections were visualized with red chromogen at 40°C and counterstained using the Mayer hematoxylin method. Sections were mounted and observed under light microscopy.

In general, immunohistochemical staining of *p53* was done with the same staining protocol of that for c-erbB-2. Immunohistochemical staining was carried out using anti-*p53* monoclonal antibody (1 : 100, clone DO-7; Dako) as the primary antibody. Sections were incubated for 1 h at room temperature. Sections were also visualized with red chromogen at 40°C and counterstained with Mayer's hematoxylin.

3) Assessment of staining

Two pathologists independently assessed the degree of staining without clinical information of the patient. For c-erbB-2, a scoring system was applied according to location and degree of completion of staining: 0 points: staining of $\leq$10% was equivalent; 1 point: incomplete membrane staining of $>$10%; 2 points: weak-to-moderate complete staining of the membrane; and 3 points: strong complete staining of the membrane. A score of $\geq$2 points was classified as positive staining (Fig. 1). A degree of staining of the nucleus of $>$10% was classified as positive

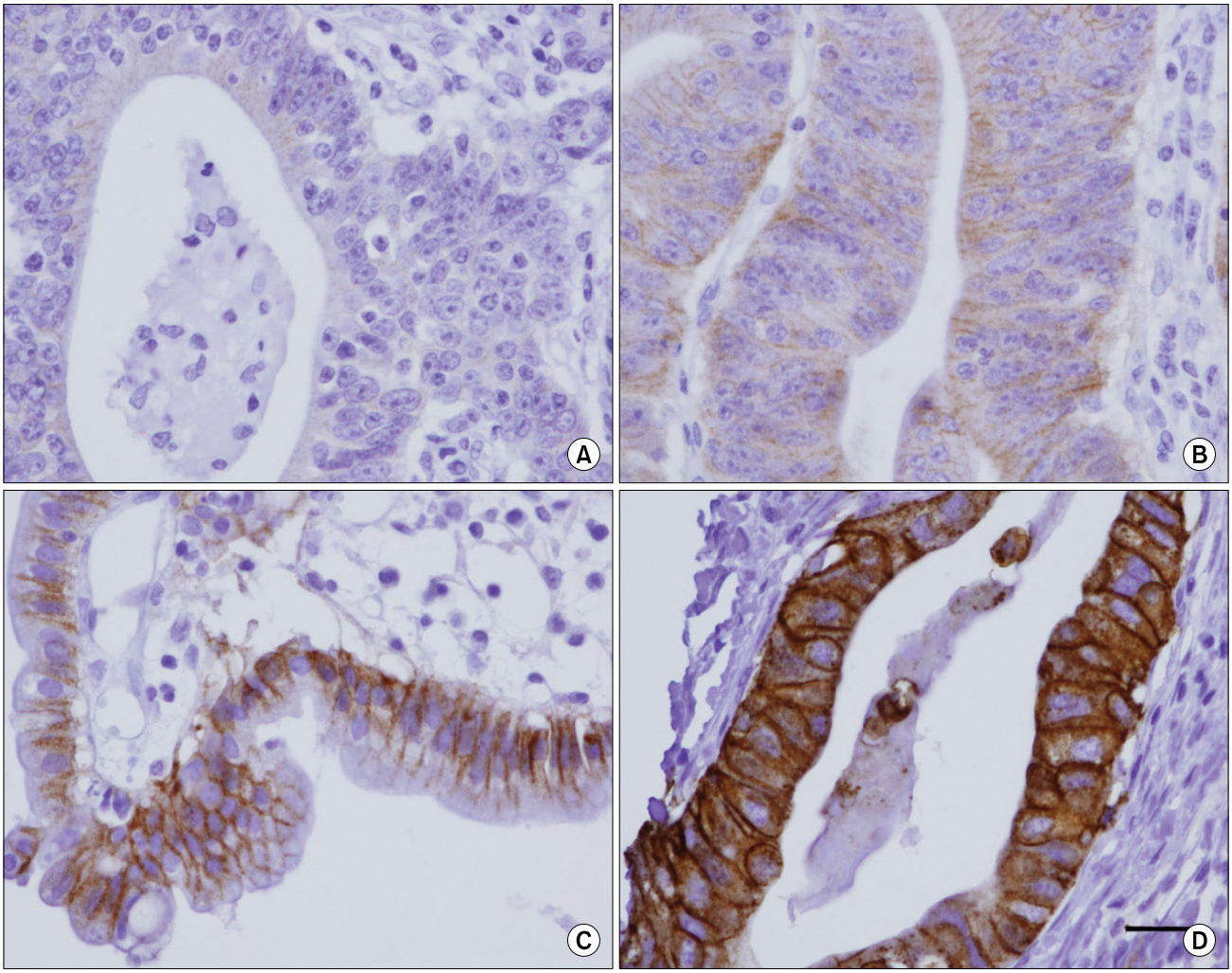

Fig. 1. Immunohistochemical staining of c-*erb*B-2. (A) No expression (0 points), (B) 1～10% expression (1 point), (C) 10～50% expression (2 points), (D) ＞50% expression (3 points). Scale bar=50 μm

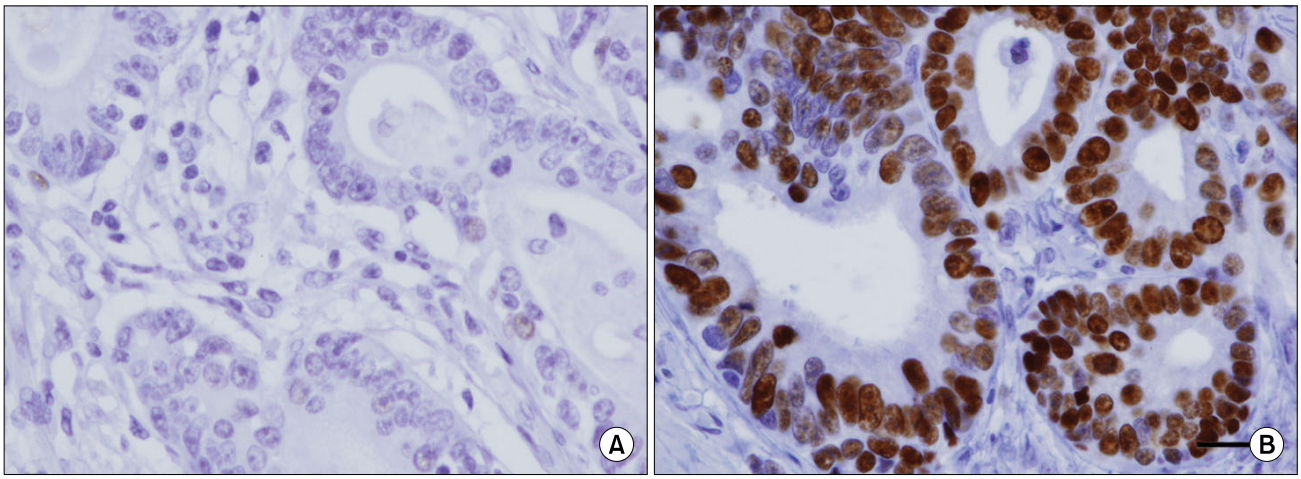

Fig. 2. Immunohistochemical staining of *p53*. (A) ＜10% expression, (B) ＞10% expression. Scale bar=50 μm.

Table 1. Immunohistochemical staining of p53 and c-erbB-2 related to clinicopathologic factors

	N	*p53*		c-erbB-2	
		Positive n (%)	P	Positive n (%)	P
Age (years)					
≤60	61	34 (55.7)	0.681	14 (23.0)	0.789
>60	64	38 (59.4)		16 (25.0)	
Sex					
Female	39	22 (56.4)	0.856	9 (23.1)	0.871
Male	86	50 (58.1)		21 (24.4)	
Tumor depth					
T1	56	26 (46.4)	0.023	8 (14.3)	0.022
T2/T3/T4	69	46 (66.7)		22 (31.9)	
Lymph node					
N0	74	40 (54.1)	0.334	11 (14.9)	0.004
N1/N2/N3	51	32 (62.7)		19 (37.3)	
Lauren class					
Intestinal	43	25 (58.1)	0.992	13 (30.2)	0.490
Mixed	37	21 (56.8)		8 (21.6)	
Diffuse	45	26 (57.8)		9 (20.0)	
Differentiation					
Differentiated	58	37 (63.8)	0.192	14 (24.1)	0.973
Undifferentiated	67	35 (52.2)		16 (23.9)	
Lymphatic invasion					
Negative	65	36 (55.4)	0.602	10 (15.4)	
Positive	60	36 (60.0)		20 (33.3)	
Venous invasion					
Negative	113	64 (56.6)	0.504	26 (23.0)	0.480
Positive	12	8 (66.7)		4 (33.3)	
Neural invasion					
Negative	74	40 (54.1)	0.334	18 (24.3)	0.919
Positive	51	32 (62.7)		12 (23.5)	

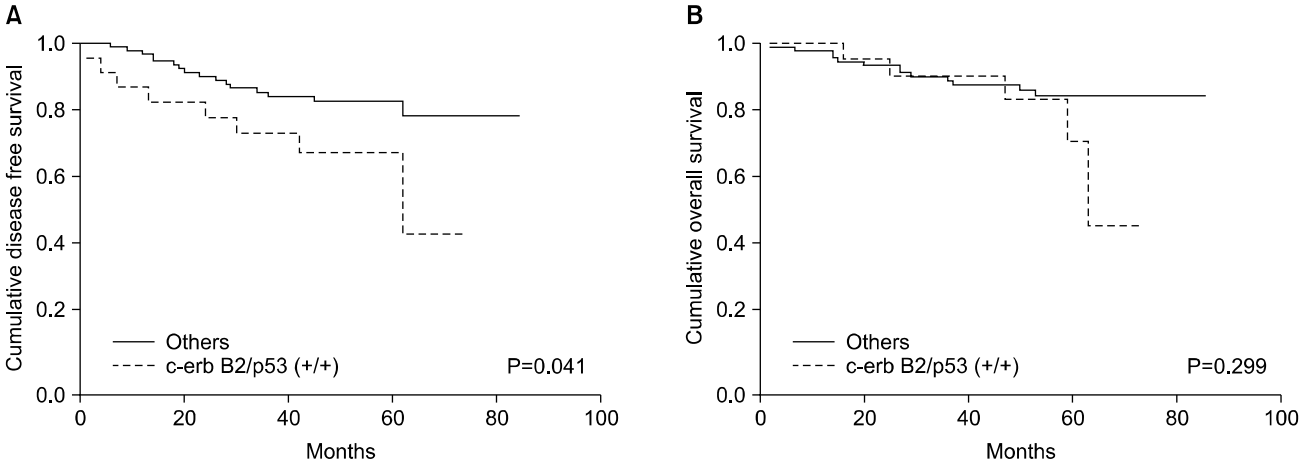

Fig. 3. Disease-free survival (A) and overall survival (B) according to expression of both c-*erb*B-2 and *p53* in all patients, P was calculated by the log rank test.

p53 staining (Fig. 2).

4) Statistical analysis

Statistical analysis was done with the Statistical Package for Social Science (SPSS Corporation, CA, USA) version 13.0. $P<0.05$ was considered significant. The correlations between expression of both proteins and clinicopathologic factors were analyzed using the chi-square test. The Kaplan-Meier method with the log-rank test was used for univariate analysis of the correlation between gene expression and survival. Factors that showed a P-value of <0.1 became candidates for multivariate analysis. Multivariate analysis for identifying the prognostic factors was done with the Cox-proportional hazard model.

Table 2. Comparison of disease-free and overall survival according to clininopathologic factors and results of immunohistochemical staining (n=125)

	N (%)	Disease-free survival			Overall survival		
		5 year (%)	Mean (month)	P	5 year (%)	Mean (month)	P
Age (years)							
≤60	61	86.3	75.2	0.048	88.8	78.6	0.067
>60	64	54.6	61.6		64.7	67.3	
Sex							
Female	39	73.7	65.7	0.388	74.0	68.6	0.144
Male	86	74.4	71.4		81.8	77.1	
Tumor depth							
T1	56	88.0	77.9	<0.001	98.1	78.6	<0.001
T2/T3/T4	69	61.8	61.5		64.2	67.8	
Lymph node							
N0	74	83.7	77.0	0.001	88.4	78.9	0.057
N1/N2/N3	51	59.9	58.7		67.8	68.2	
Lauren class							
Intestinal	43	74.8	71.3	0.505	76.4	76.0	0.401
Mixed	37	76.5	64.1		77.4	67.9	
Diffuse	45	71.8	68.8		82.1	74.9	
Differentiation							
Differentiated	58	69.2	65.7	0.903	69.6	70.5	0.590
Undifferentiated	67	76.0	70.5		84.6	76.1	
Lymphatic invasion							
Negative	65	84.7	74.5	0.001	91.2	76.1	0.031
Positive	60	62.4	62.6		68.2	70.5	
Venous invasion							
Negative	113	74.8	71.6	0.126	81.0	75.9	0.206
Positive	12	64.6	45.1		64.2	53.3	
Neural invasion							
Negative	74	82.6	75.4	<0.001	90.8	78.0	<0.001
Positive	51	60.4	59.3		63.0	66.2	
p53							
Negative	53	75.6	69.8	0.306	88.4	76.3	0.172
Positive	72	71.5	68.5		73.3	72.5	
*c-erb*B-2							
Negative	95	77.9	72.5	0.192	83.4	76.1	0.637
Positive	30	61.9	57.5		67.4	64.8	
p53/*c-erb*B-2							
Others	101	79.2	73.0	0.041	84.3	76.5	0.299
+/+	24	51.8	53.5		57.1	62.3	

RESULTS

1) Patient characteristics

The mean age of patients was 59.4±12.8 (means±SD) years. There were 86 males and 39 females. In the pathology reports, 56 patients were diagnosed with early gastric cancer, and 86 patients with advanced gastric cancer. The mean follow-up period was 48.4±10.7 months (range, 2～86 months). Twenty-four patients had recurrence and 18 patients died relating with gastric cancer. Patients with positive staining for c-erbB-2 and *p53* were 24% (30/125) and 57.6% (72/125), respectively. The percentage of positive staining for both c-erbB-2 and *p53* was 19.2% (24/125).

2) Correlation with clinicopathologic features

The positive staining of c-erbB-2 was significantly related to tumor invasion (P=0.022), lymph node metastasis (P=0.004) and lymphatic invasion (P=0.019). *p53* staining was associated with depth of invasion (P=0.023). Other factors did not correlate with staining (Table 1).

3) Survival analysis for all patients

Mean disease-free survival of 125 patients was 72.4 (65.5～75.3) months (95% confidence interval (CI)) and disease-specific overall survival was 75.2 (70.9～97.4) months (95% CI) (Fig. 3). In the univariate analysis for

Table 3. Prognostic factors by multivariate analysis (n=125)

	Beta	P	OR	95% CI
Factors for disease recurrence				
Tumor invasion				
AGCa* vs EGCa†	1.741	0.037	5.702	1.111～29.258
Neural invasion				
(+) vs (−)	1.042	0.067	2.834	0.928～8.658
Age (years)				
(>60) vs (≤60)	1.159	0.056	2.323	0.980～5.508
Factors for overall survival				
Tumor invasion				
AGCa vs EGCa	2.021	0.076	7.548	0.808～70.531
Neural invasion				
(+) vs (−)	1.190	0.091	3.288	0.828～13.058

*AGCa = advanced gastric cancer; †EGCa = early gastric cancer.

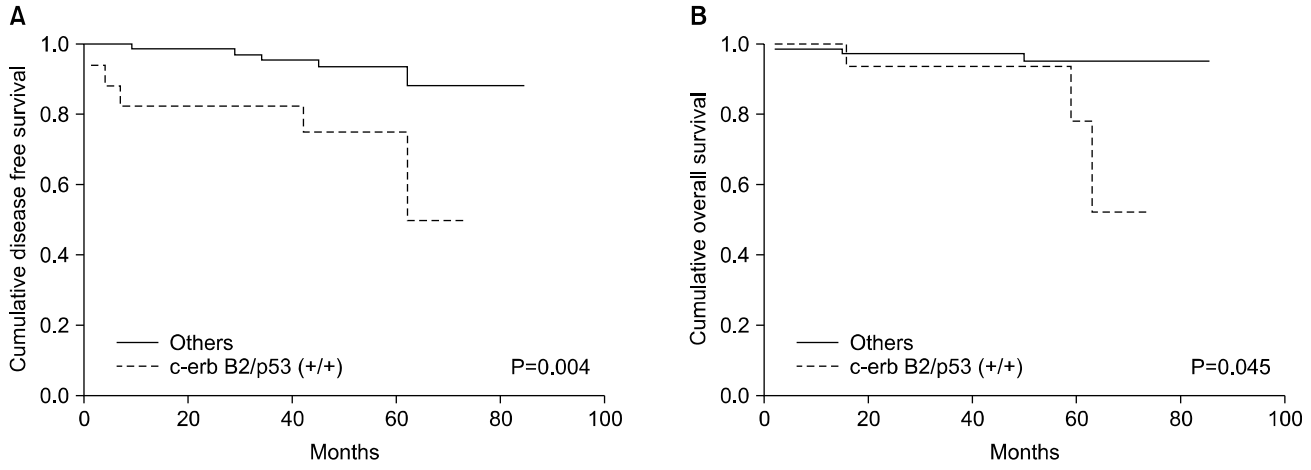

Fig. 4. Disease-free survival (A) and overall survival (B) according to expression of both c-*erb*B-2 and p53 in patients with tumors not exposed to serosa, P was calculated by the log rank test.

Table 4. Comparison of disease-free and overall survival according to clininopathologic factors and results of immunohistochemical staining in gastric cancer not exposed to serosa (n=91)

	N (%)	Disease-free survival			Overall survival		
		5 y (%)	Mean (month)	P	5 year (%)	Mean (month)	P
Age (years)							
≤60	47	91.1	78.3	0.322	94.4	82.3	0.214
>60	44	63.7	69.4		76.2	73.2	
Sex							
Female	25	79.6	72.6	0.643	89.2	72.9	0.119
Male	66	85.4	76.3		82.8	81.8	
Tumor depth							
T1	56	88.0	77.9	0.003	98.1	78.6	0.017
T2	35	69.0	67.6		73.0	74.3	
Lymph node							
N0	64	87.4	80.1	0.007	93.8	81.8	0.385
N1/N2/N3	27	66.8	64.5		78.6	75.6	
Lauren class							
Intestinal	38	78.8	73.5	0.905	79.9	76.7	0.590
Mixed	23	91.1	72.4		90.9	73.8	
Diffuse	30	77.7	74.8		94.4	80.1	
Differentiation							
Differentiated	41	78.3	71.9	0.809	77.3	73.9	0.209
Undifferentiated	50	81.6	75.4		94.3	82.4	
Lymphatic invasion							
Negative	55	88.9	78.2	0.002	98.1	79.6	0.044
Positive	36	68.6	67.9		76.1	76.1	
Venous invasion							
Negative	83	82.2	77.9	0.007	89.8	81.1	0.025
Positive	8	60.0	42.9		66.7	54.1	
Neural invasion							
Negative	70	82.8	76.1	0.003	90.8	78.8	0.010
Positive	21	71.1	65.5		63.0	73.4	
p53							
Negative	42	82.3	74.8	0.277	97.4	80.3	0.154
Positive	49	78.2	73.9		79.8	77.6	
*c-erb*B-2							
Negative	70	86.8	79.2	0.025	94.4	82.0	0.116
Positive	21	62.0	59.1		67.0	66.3	
p53/*c-erb*B-2							
Others	74	87.5	79.4	0.004	94.7	82.2	0.045
+/+	17	53.9	55.8		59.1	64.6	

survival, patients with expressions of both c-erbB-2 and *p53* gene showed a significantly low disease-free survival (P=0.041) (Table 2), although expression of each c-erbB-2 and *p53* did not show significance. The expression of both c-erbB-2 and *p53* was not predictive factor for recurrence in multivariate analysis (Table 3). In view of overall survival, immunohistochemical staining was not significant in univariate and multivariate analysis (Table 2, 3).

4) Survival analysis for patients with tumor without invasion into the serosa (pT2 and below)

Subgroup analysis was carried out for 91 patients diagnosed with pathologic T2 and below. c-erbB-2 expression (P=0.025) and simultaneous expression of c-erbB-2 and *p53* (P=0.004) were related to early recurrence in

Table 5. Prognostic factors by multivariate analysis in gastric cancer not exposed to serosa (n=91)

	Beta	P	OR	95% C.I.
Factors for disease recurrence				
Lymphatic invasion				
(+) vs (−)	2.032	0.058	7.633	0.936～62.247
p53/*c-erb*B-2				
(+/+) vs others	1.631	0.021	5.107	1.282～20.341
Venous invasion				
(+) vs (−)	1.530	0.052	4.616	0.985～21.642
Neural invasion				
(+) vs (−)	1.280	0.071	3.598	0.895～14.468
Factors for overall survival				
Neural invasion				
(+) vs (−)	1.953	0.028	7.053	1.240～40.101
p53/*c-erb*B-2				
(+/+) vs others	1.493	0.073	4.449	0.868～22.794

univariate analysis (Fig. 4). Disease-specific overall survival was also associated with expression of both c-erbB-2 and *p53* (P=0.045) (Table 4). In multivariate analysis, expression of both c-erbB-2 and *p53* was a predictive factor for recurrence (odds ratio (OR); 5.105, 95% CI: 1.282～20.341), but that was not significantly related with disease specific overall survival rate (Table 5).

DISCUSSION

We investigated the expression of c-erbB-2 and *p53* by immunohistochemical staining in gastric cancer. Analysis of the correlation of expression of these proteins with clinicopathologic results revealed that expression of both protein was associated with aggressive pathologic features. The expression of both c-erbB-2 and *p53* may be a predictive factor for recurrence, particularly in cases of relatively early stage cancer.

Recent studies have focused on predicting tumor progression by analysis of the gene expression related to carcinogenesis in gastric cancer.(*11-13*) These studies support the essential change of major genes for advance of gastric cancer. Attempts to develop a clinical application have been carried out based on these results. In connection with this, we planned to ascertain whether the expression of c-erbB-2 and *p53* genes, major oncogenes and tumor suppressor genes, are related to clinicopathologic features and the prognosis.

The c-erbB-2 oncogene is critically related with epidermal growth factor (EGF) receptor. The level of protein expressed by c-erbB-2 gene is increased in various adenocarcinoma tissue such as breast, ovary, cervix and lung; this expression is associated with aggressive clinical features and poor prognosis.(*14,15*) In gastric cancer, several studies reported that the expression rate of c-erbB-2 varied from 9% to 45%;(*5,16,17*) our result was 24%. This multiplicity of the rate of c-erbB-2 expression is thought to be due to differences in sample size. Other reports stated that c-erbB-2 expression is differed according to the histological type of gastric cancer, but correlation with prognosis is controversial.(*10,18*) In this study, c-erbB-2 expression was related to depth of invasion of tumor, lymph node metastasis and lymphatic invasion presenting aggressive features of the tumor. According to in univariate and multivariate analysis, this study did not show that patients with c-erbB-2 expressed cancer had a significantly shorter disease-free or disease-specific overall survival than that of patients who did not have c-erbB-2-expressed cancer. c-erbB-2 positive patients with *p53* expression had significantly lower disease-free survival in only univariate analysis. Particularly, in the analysis of patients who did not have serosa-exposed tumors, positive expression of c-erbB-2 and *p53* was correlated with shorter disease-free and overall survival in univariate analysis, and could be a significant

factor for predicting cancer recurrence in multivariate analysis. Expression of two genes could be one of the prognostic factors only for patients with relatively early stage tumors. This may be because the prognosis of patients with more advanced gastric cancer, serosa exposed, may be affected by other factors as well as the role in carcinogenesis of these genes.

The major functions of *p53* protein are regulation of the cell cycle and apoptosis, and repair of DNA damage. Functional abnormality of *p53* is known to be caused by mutation of the *p53* gene, including loss of heterozygosity (LOH) and DNA methylation,(*19*) which can affect the biological behavior of the tumor and therefore prognosis. Several studies have been conducted to find the correlation of abnormal expression of *p53* with prognosis in cancer of the colon,(*20*) and breast,(*7*) but the results were inconclusive. For gastric cancer, *p53* expression was 40～60% and was related to disease progression, and the degree of cellular division,(*6-8,21*) but studies reporting the correlation of *p53* expression with prognosis are lacking. In the present study, *p53* expression was 57.6% and tumor-expressed *p53* have correlated with depth of tumor invasion, but a relationship with prognosis was not evident.

In the present study, examination of c-erbB-2 or *p53* is sensible because these two genes are located on the same chromosome, and carcinogenesis by multistep mutation of genes could occur in gastric cancer. Chang et al.(*22*) reported that high expression of *p53* was strictly related to c-erbB-2 expression in breast cancer. In gastric cancer, a report in which expression of *p53* and c-erbB-2 was evaluated showed correlation between these two genes.(*23*) In the present study, of 30 tumors expressing c-erbB-2, 24 tumors (80%) also showed expression of *p53*. Statistically significant results were obtained for patients who had simultaneous expression of c-erbB-2 and *p53*: they had a poorer prognosis than that of the others. We therefore hypothesize that mutations of these two genes has a part in the carcinogenesis of gastric cancer.

New agents targeting the molecules correlated with tumor carcinogenesis have been developed. Agents focused on the c-erbB-2 and *p53* genes, as representative oncogenes and tumor suppressor genes, have been studied and transfer to the clinical setting attempted. The current experimental approach targeting *p53* aims to induce apoptosis or to prevent destruction of normal cells by chemotherapy.(*24*) Bykov et al.(*25*) reported that agents targeting a mutant *p53* gene could be effective without apparent toxic side effects, although these results were limited to an *in-vivo* pilot study. When c-erbB-2 has been used as a targeting molecule, agents such as monoclonal antibodies that can inhibit appropriate ligands combining with EGF receptors associated with c-erbB-2 expression have been developed.(*26*) In gastric cancer, there is no evidence about the clinical benefit of agents targeting specific molecules, and several clinical trials enrolling patients with non-operable gastric cancer are ongoing.(*27*) If clinical benefit can be shown in these trials, the concept of using targeting agents can be expanded into the adjuvant setting to lower recurrence after curative resection.

Adjuvant chemotherapy for gastric cancer has focused on oral fluorouracil agents. A clinical trial showed that adjuvant treatment with uracil-tegafur for patients with non-serosa-exposed tumor resected curatively could reduce recurrence and enhance survival.(*28*) The result of that trial showed that the five-year survival of these patients was 86%, and that 33% of patients complained that chemotherapy induced toxicity of grade ＞3 grade, even though the study enrolled patients with less advanced cancer. To improve prognosis and reduce chemotherapy-related toxicity, we hypothesize that these patients should be have a new adjuvant strategy according to markers expressed in the tumor and which correlated with prognosis. In the present study, simultaneous expression of c-erbB-2 and *p53* was a significant factor for prediction of recurrence and survival: using agents targetting those two molecules may merit further investigation.

REFERENCES

1) Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of eighteen major cancers in 1985. Int J Cancer 1993;54:594-606.
2) Schechter AL, Stern DF, Vaidyanathan L, Decker SJ, Drebin

JA, Greene MI, et al. The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. Nature 1984;312: 513-6.
3) Hernandez-Boussard T, Rodriguez-Tome P, Montesano R, Hainaut P. IARC p53 mutation database: a relational database to compile and analyze p53 mutations in human tumors and cell lines. International Agency for Research on Cancer. Hum Mutat 1999;14:1-8.
4) Matozaki T, Sakamoto C, Suzuki T, Matsuda K, Uchida T, Nakano O, et al. p53 gene mutations in human gastric cancer: wild-type p53 but not mutant p53 suppresses growth of human gastric cancer cells. Cancer Res 1992;52:4335-41.
5) Hilton DA, West KP. c-erbB-2 oncogene product expression and prognosis in gastric carcinoma. J Clin Pathol 1992;45: 454-6.
6) Ichiyoshi Y, Oiwa H, Tomisaki S, Sakaguchi Y, Ohno S, Maehara Y, et al. Overexpression of p53 is associated with growth pattern and prognosis in advanced gastric cancer. Hepatogastroenterology 1997;44:546-53.
7) Ostrowski JL, Sawan A, Henry L, Wright C, Henry JA, Hennessy C, et al. p53 expression in human breast cancer related to survival and prognostic factors: an immunohistochemical study. J Pathol 1991;164:75-81.
8) Starzynska T, Bromley M, Ghosh A, Stern PL. Prognostic significance of p53 overexpression in gastric and colorectal carcinoma. Br J Cancer 1992;66:558-62.
9) Yonemura Y, Ninomiya I, Ohoyama S, Kimura H, Yamaguchi A, Fushida S, et al. Expression of c-erbB-2 oncoprotein in gastric carcinoma. Immunoreactivity for c-erbB-2 protein is an independent indicator of poor short-term prognosis in patients with gastric carcinoma. Cancer 1991;67:2914-8.
10) Yonemura Y, Ninomiya I, Yamaguchi A, Fushida S, Kimura H, Ohoyama S, et al. Evaluation of immunoreactivity for erbB-2 protein as a marker of poor short term prognosis in gastric cancer. Cancer Res 1991;51:1034-8.
11) Becker KF, Keller G, Hoefler H. The use of molecular biology in diagnosis and prognosis of gastric cancer. Surg Oncol 2000;9:5-11.
12) Tamura G. Genetic and epigenetic alterations of tumor suppressor and tumor-related genes in gastric cancer. Histol Histopathol 2002;17:323-9.
13) Tamura G. Alterations of tumor suppressor and tumor-related genes in the development and progression of gastric cancer. World J Gastroenterol 2006;12:192-8.
14) Christensen JG, Schreck RE, Chan E, Wang X, Yang C, Liu L, et al. High levels of HER-2 expression alter the ability of epidermal growth factor receptor (EGFR) family tyrosine kinase inhibitors to inhibit EGFR phosphorylation in vivo. Clin Cancer Res 2001;7:4230-8.
15) Danielsen AJ, Maihle NJ. Ligand-independent oncogenic transformation by the EGF receptor requires kinase domain catalytic activity. Exp Cell Res 2002;275:9-16.
16) Allgayer H, Babic R, Gruetzner KU, Tarabichi A, Schildberg FW, Heiss MM. c-erbB-2 is of independent prognostic relevance in gastric cancer and is associated with the expression of tumor-associated protease systems. J Clin Oncol 2000;18: 2201-9.
17) Jain S, Filipe MI, Gullick WJ, Linehan J, Morris RW. c-erbB-2 proto-oncogene expression and its relationship to survival in gastric carcinoma: an immunohistochemical study on archival material. Int J Cancer 1991;48:668-71.
18) Tateishi M, Toda T, Minamisono Y, Nagasaki S. Clinicopathological significance of c-erbB-2 protein expression in human gastric carcinoma. J Surg Oncol 1992;49:209-12.
19) Hollstein M, Rice K, Greenblatt MS, Soussi T, Fuchs R, Sorlie T, et al. Database of p53 gene somatic mutations in human tumors and cell lines. Nucleic Acids Res 1994;22:3551-5.
20) Scott N, Sagar P, Stewart J, Blair GE, Dixon MF, Quirke P. p53 in colorectal cancer: clinicopathological correlation and prognostic significance. Br J Cancer 1991;63:317-9.
21) Ioachim E, Goussia A, Stefanou D, Agnantis NJ. Expression of p53 protein in gastric cancer: an immunohistochemical study with correlation to proliferative activity. Anticancer Res 1997;17:513-7.
22) Chang K, Ding I, Kern FG, Willingham MC. Immunohistochemical analysis of p53 and HER-2/neu proteins in human tumors. J Histochem Cytochem 1991;39:1281-7.
23) Sasano H, Date F, Imatani A, Asaki S, Nagura H. Double immunostaining for c-erbB-2 and p53 in human stomach cancer cells. Hum Pathol 1993;24:584-9.
24) Bouchet BP, de Fromentel CC, Puisieux A, Galmarini CM. p53 as a target for anti-cancer drug development. Crit Rev Oncol Hematol 2006;58:190-207.
25) Bykov VJ, Issaeva N, Shilov A, Hultcrantz M, Pugacheva E, Chumakov P, et al. Restoration of the tumor suppressor function to mutant p53 by a low-molecular-weight compound. Nat Med 2002;8:282-8.
26) Baxevanis CN, Sotiropoulou PA, Sotiriadou NN, Papamichail M. Immunobiology of HER-2/neu oncoprotein and its potential application in cancer immunotherapy. Cancer Immunol Immunother 2004;53:166-75.
27) Ohtsu A. Chemotherapy for metastatic gastric cancer: past, present, and future. J Gastroenterol 2008;43:256-64.
28) Nakajima T, Kinoshita T, Nashimoto A, Sairenji M, Yamaguchi T, Sakamoto J, et al. Randomized controlled trial of adjuvant uracil-tegafur versus surgery alone for serosa-negative, locally advanced gastric cancer. Br J Surg 2007;94:1468-76.