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The Prognostic Significance of c-erbB-2 and p53 Protein Expressions in Gastric Carcinoma

—A Multivariate Analysis of Prognostic Factors—

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152 curative gastrectomy specimens from patients with gastric carcinoma were examined in an attempt to assess the prognostic value of c-erbB-2 and mutant p53 protein expressions. The labeled streptavidin-biotin method was applied to routinely fixed and paraffin-embedded tissue sections, using the polyclonal and monoclonal antibodies against the c-erbB-2 protein and the mutant form p53 protein, respectively. in this examination, staining of c-erbB-2 protein was found in 9.2% of these carcinomas. The c-erbB-2 stained tumors were significantly associated with the tumors whose diameters were smaller than 5cm, and were more likely to be associated with serosal invasion and nodal involvement than the unstained ones. However, there was little association between staining of c-erbB-2 protein and clinicopathologic findings such as age, sex, location, histology, gross type, lymph node status, depth of invasion, and stage. The survival analysis of 104 patients with stage III gastric carcinoma revealed no significant association between c-erbB-2 staining status and survival duration. The 5-year survival rates of the c-erbB-2 positive group and its negative group were 21% and 28%, respectively. Positive p53 protein expression was observed in 46% of 152 carcinomas. There was no significant association between p53 expression and parameters such as age, sex, location, histology, gross type, and size. The p53 stained tumors were more likely to be associated with lymph node metastasis, serosal invasion than p53 unstained ones ; but this did not reach significance. The 5-year survival rates of the p53 positive group and counter part group were 27% and 31%, respectively. Although the difference among 5-year survival rates was not significant, it was noticeable that the 5-year survival rate was relatively low in the group of patients with positive p53 protein expression, whose tumor was smaller than 5 cm in diameter. It is believed that there was a higher incidence of lymph node metastasis when p53 protein expression occurred in tumors smaller than 5cm in diameter. This suggests that p53 protein has a possible role in lymph node metastasis. When the status of c-erbB-2 and p53 protein tissue expression and clinicopathologic factors(including such known prognostic factors as lymph node status and depth of invasion) were simultaneously analyzed by means of multiple linear regression, the results of our study

showed that the expressions of product of c-erbB-2 oncogene or p53 tumor supressor gene had little prognostic significance in gastric carcinoma.

Key Words : *Gastric carcinoma, Prognostic factor, c-erbB-2, p53*

INTRODUCTION

The c-erbB-2 oncogene is a member of the tyrosine kinase oncogene family located in chromosome 17q and codes for a 185 Kd transmembrane glycoprotein similar to epidermal growth factor receptor(Akiyama et al., 1986). This gene is believed to control the differentiation or growth of some types of adenocarcinomas. In breast carcinoma, c-erbB-2 amplification correlates well with immunohistochemical staining for the protein production and prognostic value(Slamon et al., 1989),

The p53 gene is located in chromosome 17p. The normal p53 gene acts as a recessive oncogene, while p53 gene mutations change the apparent function to that of a dominant oncogene (Baker et al., 1990). The mutations of the p53 gene which have been considered the common genetic abnomalities in various kinds of human cancers are related to tumorigenicity and metastastic potential in colorectal cell lines (Weinberg, 1991 ; Baker et al., 1990).

Expressions of c-erbB-2 and mutant p53 proteins have been observed in some gastric carcinomas but reports of their prognostic value are conflictual. In this study we investigated c-erbB-2 and p53 protein expressions and their relation to the clinical outcomes of patients with gastric carcinomas, including prognosis.

MATERIALS AND METHODS

For our investigation, 152 cases of well preserved formalin-fixed and paraffin-embedded specimens were taken from 171 cases who underwent gastrectomy combined with lymph node dissection from July to December 1987 at Seoul national university hospital in Korea. 88 men and 64 women with a mean age of 54 years(20 to 79 years) were studied. Of these 152 patients, seven had early gastric cancer, 22 and 26 had UICC stage I and stage II, respectively ; 34 and 70 had stage IIIa and IIIb, respectively.

For c-erbB-2 protein staining, deparaffinized 5 micron sections were covered with normal rabbit serum for 25 minutes. The primary antibody, polyclonal rabbit anti-human immunoglobulin (Dakopatts, Copenhagen, Denmark) at a concentration of 1 : 200 in TBS, was then applied and left overnight. Following TBS washing, the sections were incubated with a secondary antibody, biotinylated rabbit anti-rabbit immunoglobulin(Dakopatts), for 10 minutes, then with streptavidin-biotin-peroxidase complex-(Dakopatts) for 10 minutes, and then the color was developed with 3-,3'-diaminobenzidine(DAB) Finally, the slides were counterstained with hematoxylin, dehydrated, and mounted in routine fashion.

For staining mutant p53 protein, the same tissue was treated in a similar pattern by using 1 : 50 monoclonal mouse anti-human immunoglobulin, DO-7(Novocastra, England) for a primary antibody incubated for 3 hours and biotinylated rabbit anti-mouse immunoglobulin(Dakopatts) for a secondary antibody.

Negative controls were done by running parallel sections with the serum instead of the primary antibody. A known positive case of human breast cancer containing tumors cells more than 50% was used as a positive control. The tumors that showed cell membrane staining more than 10% were considered positive for the over-expression of c-erbB-2 protein, while those which showed definite nuclear staining more than 10% were regarded as positive for the expression of mutant p53 protein.

Our survival data shows that 85 were dead, 36 were alive and 31 were lost to follow up(follow up rate=80%), among 152 patients with follow-up times of more than 5 years(median follow up duration=32 months). The tumors were classified according to such variables as age, sex, tumor locations, histology, gross types, depth of invasion, regional lymph node status, size of tumor, and stages. For the statistical analysis of data, the chi-

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square test was carried out. For the survival analysis of 104 patients with stage III gastric carcinoma, Kaplan-Meier method was used. The log rank test was used to compare the differences of survival among the subgroups. In addition, Weibull's model was applied for the multivariate analyses of the prognostic factors by multiple linear regression analysis. The statistical procedures "PROC LIFETEST" and "PROC LIFEREG" were performed for the above analysis by using the package "SAS for PC."

RESULTS

Staining for c-erbB-2 protein occurred in 14(9.2%) turmors whose reaction was localized in cell membranes. Turmors smaller than 5cm in diameter had more frequent evidence of c-erbB-2 staining(17%) than those larger than 5cm in diameter(5%). c-erbB-2 staining occurred more frequently when the tumor metastasized to the lymph nodes or invaded the serosa. However, there was no statistically significant association between c-erbB-2 staining and such clinicopathologic parameters as age, sex, location, histology, gross type, lymph node status, depth of invasion, and stage. Positive p53 staining was seen in the nuclei of 70(46%) tumors. p53 positivity was more frequently observed when the tumors metastasized to the lymph nodes or invaded the serosa. There was no statistically significant association between p53 expression and the above clinicopathologic parameters, although a definite trend was seen between nuclear p53 positivity and such parameters as lymph node status, serosal status, or tumor stage(Table 1).

Survival analyis of patients with stage III gastric carcinoma(n=104) revealed no association between the staining status of c-erbB-2 or p53 protein

Table 1. Association of p54 and c-erbB-2 expression* with clinicopathologic findings in gastric cancer(n=152)

	No. of case	No. of Expression p53	No. of Expression c-erbB-2		No. of case	No. of expression p53	No. of expression c-erbB-2
Age				Tumor location-1			
<40	17	7(41%)	2(12%)	Upper 1/3	7	4(57%)	0
50-49	32	9(28%)	2(6%)	Mid 1/3	34	14(41%)	3(9%)
50-59	54	28(52%)	6(11%)	Lower 1/3	76	36(47%)	11(15%)
60-69	34	19(56%)	3(9%)	Combined	35	16(46%)	0
70-79	15	7(47%)	1(7%)	Tumor location-2			
Sex				Lesser curv	93	42(45%)	8(9%)
Male	88	42(48%)	9(10%)	Greater curv	27	10(37%)	3(11%)
Female	64	28(44%)	5(8%)	Anterior wall	13	7(54%)	2(15%)
Histology				Posterior wall	7	5(71%)	0
Well	17	10(59%)	2(12%)	Encircling	12	6(50%)	1(8%)
Moderate	42	19(45%)	5(12%)	Macroscopic type			
Poor	69	33(48%)	6(9%)	Borrmann I	1	1(100%)	1(100%)
Signet	9	3(33%)	1(11%)	Borrmann II	32	18(56%)	2(6%)
Mucinous	12	4(33%)	0	Borrmann III	96	44(46%)	11(11%)
Depth of invasion				Borrmann IV	16	6(38%)	0
Serosa(−)	30	11(37%)	2(7%)	EGC	7	1(14%)	0
Serosa(+)	122	59(48%)#	12(10%)#	Tumor stage			
Lymph node meta.				I	22	8(36%)	2(9%)
N0	44	17(39%)	3(7%)	II	26	12(46%)	1(4%)
N1	29	13(45%)	3(10%)	IIIa	34	14(41%)	3(9%)
N2	79	40(51%)#	8(18%)#	IIIb	70	36(51%)	8(11%)
Tumor size							
<5cm	53	25(47%)	9(17%)				
≥5cm	99	45(45%)	5(5%)**				

* p53 and c-erbB-2 expressed by immunohistochemical staining of paraffin block
** Statistically significant association was observed(p=0.015) by Chi-square test
# Trend of association without statistical significance

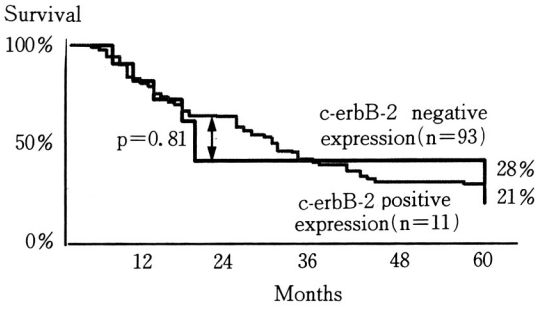

Fig. 1. Survival curve of stage Ⅲ gastric cancer patients according to c-erbB-2 protein expression status.

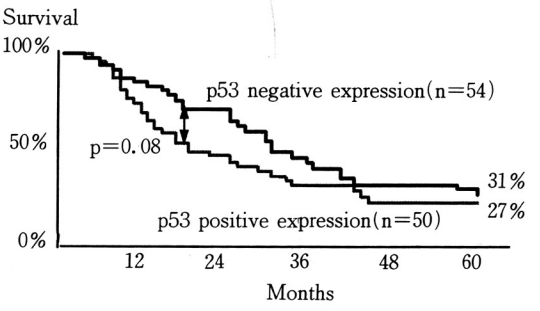

Fig. 2. Survival curve of stage Ⅲ gastric cancer patients according to p53 protein expression status.

Table 2. Result of univariate survival analysis* in stage Ⅲ gastric cancer(n=104)

Variables	Number of cases	Median follow up (months)	Survival		Log rank test
			Median (months)	5YSR	
entire oase	104	20	18	17%	
c-erbB-2(−)	93	20	29	28%	
c-erbB-2(+)	11	18	18	21%	NS
p53(−)	54	24	33	31%	
p53(+)	50	16	20	27%	NS
p53(+)erbB-2(+)	9	16	16	27%	
p53(−)erbB-2(−)	52	22	30	27%	NS
size <5cm	31	22	33	35%	
size ≥5cm	73	17	26	27%	NS
p53(−) size ≥5cm	40	17	29	49%	
p53(+) size ≥5cm	33	18	25	26%	NS
p53(−)serosa(−)N1	4	61	60	50%	
p53(+)serosa(−)N1	5	60	60	58%	
p53(−)serosa(+)N2	29	17	18	10%	
p53(+)serosa(+)N2	22	14	25	18%	NS
p53(−) size <5cm	14	33	39	41%	
p53(+) size <5cm	17	16	16	32%	p=0.04
N1	24	39	58	52%	
N2	79	16	24	21%	p=0.02
serosa(−)	36	61	60	58%	
serosa(+)	68	11	24	15%	p=0.01

* Kaplan-Meler method survival rates compared by log-rank test
NS : statistically not significant

and survival time. The 5-year survival rates of the c-erbB-2 positive group and its negative group were 21% and 28%(median survival : 18 and 29 months), respectively (Fig. 1). The 5-year survival rates of the p53 positive group and its negative group were 27% and 31%(median survival : 20 and 33 months), respectively (Fig. 2). A significantly low 5-year survival rate was observed in patients who showed positive p53 expression in tumors smaller than 5cm in diameter. Among these patients, the 5-year survival rates of the p53 positive group and its negative group were 32% and 41%, respectively-(Fig. 3). This difference in survival rates was due to the higher incidence of lymph node metastasis in the p53 positive group, especially in the patients

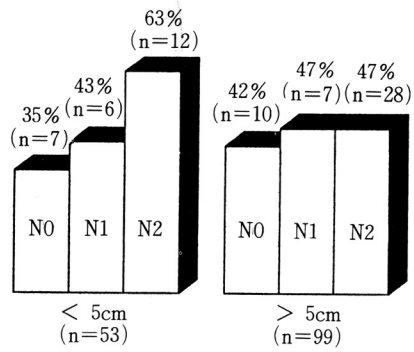

Fig. 4. Expression of p53 protein subgrouped by tumor size and lymph node status.

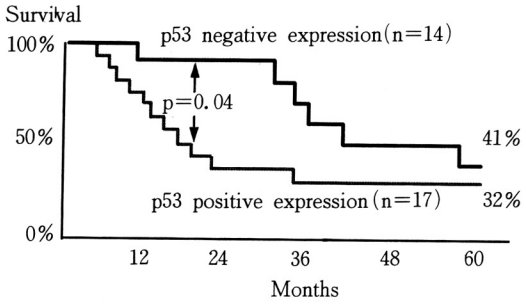

Fig. 3. Survival curve of stage Ⅲ gastric cancer patients with tumors smaller than 5cm according to p53 protein expression status.

whose tumor was smaller than 5cm in diameter(Fig. 4). However, the difference was not statistically significant when the nodal and the serosal status were controlled(Table 2).

The multivariate analysis demonstrated that serosal invasion and nodal status were significant prognostic factors. By contrast, it showed that c-erbB-2 and p53 protein expression status, like other clinicopathologic parameters, were of little independent prognostic value(Table 3).

DISCUSSION

In this immunohistochemical study of gastric can-

Table 3. Result of multivariate analysis* in stage Ⅲ gastric cancer(n=104)

Variables	Categories	DF	Chi-sq	P-value
1 LYMPH NODE	NO/N1/N2	2	25.559	0.0001**
2 DEPTH	Serosa(−)/Serosa(+)	1	10.802	0.0010**
3 LOCATION	A/M/C/Combine	3	11.153	0.0609
4 AGE	<40, 40, 50, 60, 70	4	7.710	0.1028
5 p53	+/−	1	1.646	0.1995
6 HISTOLOGY	W/M/P/Sig/Mucin	4	5.777	0.2164
7 SEX	M/F	1	1.214	0.2705
8 p53 & erbB2	N, N/N, P/P N/P, P***	3	2.282	0.5160
9 c-erbB2	+/−	1	0.409	0.5223
10 LOCATION-B	Ant/Post/Lc/Gc/Encircle	4	0.792	0.9395
11 SIZE	≥5cm/<5cm	1	0.165	0.6837
12 GROSS TYPE	BORRMANN Ⅰ/Ⅱ/Ⅲ/Ⅳ	3	0.942	0.6243

* multiple linear regression analysis
** statistically significant prognostic factor
*** N : negative, P : positive

cer, there were 9.2% of carcinomas with evidence of c-erbB-2 protein in which the reaction was localized to cell membranes, and 46% of carcinomas with evidence of p53 protein in which the reaction was localized to nuclei. These values do not differ so much from reports of other studies(Hilton & West, 1992 ; Ninomiya et al., 1990 ; Starzynska et al., 1992 ; Martin et al., 1992). Immunohistochemically, minor differences in the staining positive rate between the studies may be derived from the methods of staining and interpretation(Domagala et al., 1993). But our previous study of the breast cancer tissues with same c-erbB-2 staining method showed the similar positive rate as other reports.

The trends of c-erbB-2 and p53 expressions associating lymph node metastasis and serosal invasion were observed as in some other studies(Ninomiya et al., 1990 ; Yonemura et al., 1991 ; Martin et al., 1992). This suggests that c-erbB-2 and p53 protein expressions are related to biologic behaviour and that they may play a role during the progression of the gastric carcinoma, though they were statistically insignificant in our study.

A number of studies have correlated c-erbB-2 protein expression with prognosis(Yonemura et al., 1991 ; Takamura et al., 1993), while others have not(Tsuburaya et al., 1992 ; Ohguri et al., 1993). In our study, c-erbB-2 protein overexpression in gastric carcinomas did not show a significant relationship to poor prognosis. On the contrary, poorer prognosis was observed in the p53 protein expression group as in other reports(Starzynska et al., 1992 ; Martin et al., 1992), but it did not reach statistical significance. We found a poor prognosis in patients with positive p53 protein expression with tumor diameters of smaller than 5cm. It may derived from the dependency of p53 expression to the lymph node status(Kaetsu, 1992). Further evaluation with a larger number of cases is necessary.

The result of our multivariate analysis suggests that detecton of c-erbB-2 and p53 protein expression immunohistochemically has little value in estimating a prognosis for patients with gastric carcinomas. We may say that the two conventional prognostic factors(Kim et al., 1992 ; Maruyama, 1986) ; lymph node status and depth of invasion, are still the most valuable prognostic factors in gastric carcinoma.

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