10.1053.paor.1999.0171 available online at http://www.idealibraty.com on IDEAL

ARTICLE

Expression of c-erbB-2 Oncoprotein in Gastric Carcinoma: Correlation with Histopathologic Characteristics and Analysis of Ki-67

Ayşe DURSUN, Aylar POYRAZ, Betül CELIK, Gülen AKYOL

Department of Pathology, Gazi University Medical School Besevler, Ankara, Turkey

Amplification and overexpression of the c-erbB-2 gene has been demonstrated in several tumors and thought to be important determinants of biologic behaviors of carcinomas. In this study, correlation between c-erbB-2 expression und histopathologic parameters, including proliferative activity of gastric carcinomas was evaluated. Paraffin-embedded tissue sections from 62 patients who underwent curative resection of gastric carcinoma were analyzed immunohistochemically for the expression of c-erbB-2 and Ki-67. Strong membrane staining for c-erbB-2 was detected in 11 of 62 gastric carcinomas (17,7%) and no positive reaction was evident in noncancerous tissue. The incidence of c-erbB-2 positivity in intestinal type carcinomas (24,3%) was higher than that of diffuse type carcinomas (4,76%). Positive staining for c-erbB-2 was present in one of the 9 (11,1%) early gastric carcinomas and 10 of 53 (18,8%) advanced gastric carcinomas. However, no statistically significant relationships were found between c-erbB-2 expression and histopathologic type, depth on invasion, the tumor size or lymph node metastases. Among the metastatic lymph nodes, 3 were positively stained with c-erbB-2 whereas the primary tumors of two cases had been found to be negative. Additionally, no correlation was found between c-erbB-2 reactivity and proliferative activity of carcinoma cells. The results suggest that expression of c-erbB-2 protein may occur selectively in intestinal type of gastric carcinomas. However, c-erbB-2 expression is not a reliable marker of malignant potential in gastric carcinomas. (Pathology Oncology Research Vol 5, No 2, 104–106, 1999)

Key words: c-erbB-2, Ki-67, gastric carcinomas

Introduction

The c-erbB-2 gene is localized on chromosome 17 at q21 and encodes a 185-kDa cell-surface glycoprotein which is closely related to the epidermal-growth factor receptor (EGFR) but biologically distinct from it.[17] Gene amplification or overexpression of c-erbB-2 and its relation with prognosis, has been reported for several tumors, including breast, lung, salivary gland, urinary bladder tumors.[16] Several studies mentioned the genetic background of gastric carcinoma, and a number of genetic alterations, including amplification of c-erbB-2 gene or overexpression of its protein, have been reported.[3,8]

In this study, the expression of c-erbB-2 protein was investigated in gastric carcinomas and the correlation between c-erbB-2 expression, histopathological features and proliferative activity of gastric carcinomas was evaluated.

Material and Methods

Sixty-two cases with gastric carcinomas who had undergone gastrectomy at Gazi University Hospital between the years of 1991 and 1996 were reevaluated. Histological classification of the tumors were done according to Lauren[6] and WHO. Tumor size, depth of invasion, lymph node metastasis and adjacent noncancerous mucosal changes were determined. Sixty-two carcinomas and 9 lymph nodes with metastasis were used for immunohistochemical study of cerbB-2 protein. We studied proliferation activity of 62 gas-

Received: Dec 22, 1998; *accepted:* Jan 18, 1999

Correspondence: Ayşe DURSUN, M.D., Turgut Reis Caddesi 16/8, Mebusevleri Tandogan, 06580 Ankara, Turkey; Tel: +90 312 2137494, fax: +90 312 2129908

Presented in XXIInd Congress International Academy of Pathology and 13th World Congress of Academic and Environmental Pathology, Oct, 1998, Nice, France. This project was supported by Gazi University Investigation Found.

 1219-4956/99/020104+03 $ 12.00/0

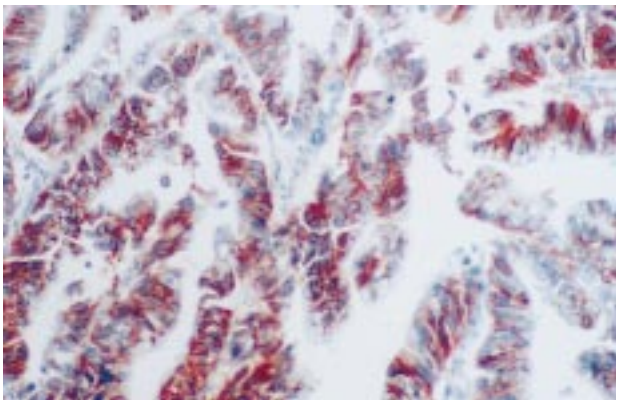

Figure 1. *Strong membrane immunoreactivity with c-erbB-2 in gastric carcinoma of intestinal type (SAB-peroxidase-DAB x200)*

tric carcinomas by Ki-67 immunostaining. Formalin-fixed, paraffin-embedded tissues were used for immunohistochemical staining. Reactions with anti-c-erbB-2 monoclonal antibody (CB 11), and anti-Ki-67 monoclonal antibody (MIB-1) were performed using the streptavidin biotin peroxidase method along with AEC (3-amino-9-ethylcarbazole in N,N-dimethyl-formamide) as chromogen.

Overexpression of the c-erbB-2 protein is defined as staining of tumor cell membranes. When only cytoplasmic staining was evident, the case was judged as negative. Only the cells showing nuclear staining were assumed positive for Ki-67 and scored as (+) 0-33%, (++) between 34-66%, (+++) between 67-100%. Statistical analysis was performed using chi-square and Fisher chi-square tests.

Table 1. **Correlation between c-erbB-2 positivity and clinicopathologic findings**

		C erbB-2 staining		
		Negative	*Positive*	
Tumor size	< 5	23	6	(p=0.813) NS
	≥ 5	28	5	
Depth of invasion	early (m, sm)	8	1	(p=0.927) NS
	advanced (mp, s)	43	10	
Histologic type (Lauren)	intestinal	31	10	(p=0.056) NS
	diffuse	20	1	
Histologic type (WHO)	well-moderately differentiated	21	7	(p=1.047) NS
	poorly differentiated	30	4	
Nodal involvement	positive	32	7	(p=0.956) NS
	negative	19	4	

NS: Not significant

Results

There were 11 (17,7%) carcinomas showing membranous c-erbB-2 protein expression (*Figure 1*). In 4 (36,3%) of 11 positive cases cytoplasmic staining was noticed as well. Weak cytoplasmic staining of adjacent noncancerous gastric epithelium and the areas of intestinal metaplasia were determined in only 2 cases. Positive staining for c-erbB-2 protein was detected in 24,3% (10 of 41) of intestinal type carcinomas. Conversely, among 21 cases of diffuse type carcinomas positive staining for c-erbB-2 protein was observed in only 1 case (4,7%). Although a higher incidence of c-erbB-2 expression was found in intestinal type carcinomas than in the diffuse type carcinomas, this was not statistically significant. 9 cases were early carcinomas in which the lesions were limited to the mucosa or submucosa whereas 53 cases were advanced carcinomas. The positivity rate of c-erbB-2 was 11,1% in early carcinomas and 18,8% in advanced carcinomas. However, there was no significant association between positivity of c-erbB-2 and depth of invasion, tumor size, lymph node metastases *(Table 1)*. c-erbB-2 protein was detected in 3 of 9 metastatic lymph nodes and primary tumors were negative in 2 of these 3 cases.

Additionally, c-erbB-2 reactivity was not found to be correlated with Ki 67 positivity *(Table 2)*.

Discussion

Immunohistochemical membranous staining incidence of c-erbB-2 protein has been reported to be 10-35% in gastric carcinomas.[1,3,8,9,14,18] In the current study a positive rate of 17,7% was achieved for c-erbB-2 immunoreactivity. Weak cytoplasmic staining of adjacent noncancerous mucosa and areas of intestinal metaplasia was evaluated as negative. Only cytoplasmic staining of c-erbB-2 has been reported in gastric carcinomas by several authors.[7,12] This diffuse type cytoplasmic immunoreactivity could be explained by the presence of oncoprotein in the mitochondrial cristae, nuclear membrane, endoplasmic reticulum or Golgi apparatus, as has been reported in immunoelectron microscopic studies.[10] It is interesting that cytoplasmic staining has not been detected in intestinal type of gastric carcinomas in which amplification and overexpression of c-erbB-2 have been demonstrated more frequently.[5,7,12] The biological and clinical significance of cytoplasmic staining is still controversial. In fact several previous studies have shown

Table 2. **Relationship between Ki-67 scores and c-erbB-2 positivity**

c-erbB-2	*Ki-67*				
	Negative	*Positive*			*Total*
		+	++	+++	
Positive	4	4	2	1	11
Negative	24	14	6	7	51
Total	28	18	8	8	62

*X^2 p=0.89 NS

that membrane staining is the only staining pattern which correlates with gene amplification.[8,9]

The incidence of c-erbB-2 expression in intestinal type – well differentiated carcinomas has been shown to be higher than in diffuse type.[7,8,9,15] Although we demonstrated a higher incidence of staining with anti-c-erbB-2 in intestinal type carcinomas than in the diffuse type; the difference was not significant statistically.[1,11,12]

Previously, the authors reported that the incidence of c-erbB-2 positivity in advanced carcinomas were higher than in early carcinomas.[9,18] In the current study we did not find any correlation between c-erbB-2 expression and the depth of invasion, similar to the various studies.[12,15] In addition we were unable to show any correlation between c-erbB-2 expression and the other parameters including tumor size, lymph node metastasis, in agreement with the literature.[8,15] However, the correlation of c-erbB-2 expression with the lymph node metastasis in well differentiated adenocarcinomas was confirmed by Motojima et al.[11] In contrast Mizutari et al and Ohguri et al reported no association between c-erbB-2 expression and lymph node metastasis in gastric carcinomas. However, the incidence of c-erbB-2 expression in metastatic lymph nodes has been reported to be significantly higher than that in primary tumors.[9,12] We observed immunoreactivity of c-erbB-2 in 3 of 9 metastatic lymph nodes and 2 cases showed positive staining in metastatic lymph nodes but not in the primary tumors. This observation was also reported by Chariyalertsak et al.[1] These results support that oncogene alterations might occur during tumor progression and metastasis of gastric carcinoma.

Consistent with several studies we found no correlation between c-erbB-2 immunoreactivity and the proliferative activity.[2,4]

Amplification and expression of c-erbB-2 in gastric carcinomas have been related to their aggressive clinical behaviour and poor survival rates by some authors.[7,9,13,14,15,18] Although it has been reported that the immunoreactivity of c-erbB-2 was not associated with altered survival in patients with poorly differentiated carcinomas by some others.[11,12]

In conclusion, expression of c-erbB-2 may occur at a significant frequency in gastric carcinogenesis, especially in intestinal type. Since the expression of c-erbB-2 is unrelated to the clinocopathologic features of the tumor, it may have a limited effect on the biologic behavior of gastric carcinoma.

References

1. *Chariyalertsak S, Sugano K, Ohkura H, et al:* Comparison of c-erbB-2 oncoprotein expression in tissue and serum of patients with stomach cancer. Tumor Biol 15:294-303, 1994.
2. *Chen BF, Marrogi AJ, Freeman SM, et al:* Gastric carcinoma recept issues in prognostic factors. J Lou State Med Soc 147:138-145, 1995.
3. *Falck VG, Gullick WJ:* C-erbB-2 oncogene product staning in gastric adenocarcinoma: An immunohistochemical study. J. Pathol 159:107-111, 1989.
4. *Flejou JF, Paraf F, Muzeau F, et al:* Expression of c erb B-2 oncogene product in Barrett's adenocarcinoma: Pathological and prognostic correlations. J Clin Pathol 47:23-26, 1994.
5. *Kameda T, Yasui W, Yoshida K, et al*: Expression of ERBB2 in human gastric carcinomas: relationship between p185ERBB2 expression and the gene amplification. Cancer Res 50:8002-8009, 1990.
6. *Lauren P:* The two histological main types of gastric carcinoma: diffuse and so-called intestinal type carcinoma. Acta Pathol Microbiol Scand 64:31-49, 1965.
7. *Lee EY, Cibull ML, Strodel WE, et al:* Expression of HER-2/neu oncoprotein and epidermal growth factor receptor and prognosis in gastric carcinoma. Arch Patol Lab Med 118:235-239, 1994.
8. *Lemoine NR, Jain S, Silvestre F, et al:* Amplification and overexpression of the EGF receptor and c-erbB-2 proto-oncogenes in human stomach cancer. Br J Cancer 64:79-83, 1991.
9. *Mizutani T, Onda M, Tokunaga A, et al:* Relationship of c-erbB-2 protein expression and gene amplification to ínvasion and metastasis in human gastric cancer. Cancer 72:2083-2088, 1993.
10. *Mori S, Akiyama T, Miorishita Y, et al:* Light and electron microscopical demonstration of c-erbB-2 gene product like immunoreactivity in human malignant tumors. Virchows Arch B Cell Pathol 54:8-15, 1987.
11. *Motojima K, Furui J, Kohara N, et al:* erb-2 expression in well-differentiated adenocarcinoma of the stomach predicts shorter survival after curative resection. Surgery 11S: 349-354, 1994.
12. *Ohguri T, Sato Y, Koizumi W, et al:* An immunohistochemical study of c erb B-2 protein in gastric carcinoma and lymph node metastasis: Is the c-erbB-2 protein really a prognostic indicator? Int J Cancer 53:75-79, 1993.
13. *Orita H, Maehara Y, Emi Y, et al:* C-erbB-2 expression is predictive for lymphatic spread of clinical gastric carcinoma. Hepatogastroenterology 44:294-298, 1997.
14. *Tsugawa K, Fushida S, Yonemura Y:* Amplification of the c-erbB-2 gene in gastric carcinoma: Correlation with survival. Oncology 50:418-425, 1993.
15. *Uchino S, Tsuda H, Maruyama K, et al:* Overexpression of c-erbB-2 protein in gastric cancer. Cancer 72:3179-3184, 1993.
16. *Wright C, Agnus B, Nicholson S, et al:* Expression of c-erbB-2 oncoprotein; a prognostic indicator in human breast cancer. Cancer Res. 49:2087-2090, 1989.
17. *Yamamoto T, Ikawa S, Akiyama T:* Similarity of protein encoded by the human c-erbB-2 gene to epidermal growth factor receptor. Nature 319:230-234, 1986.
18. *Yonemura Y, Ninomiya I, Ohoyama S, et al:* Expression of c-erbB-2 oncoprotein in gastric carcinoma. Cancer 67:2914-2918, 1991.