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MULTIPLE GENETIC EXPRESSION ABNORMALITIES IN GASTRIC CANCER

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By means of immunohistological technique, the expressions of p53, c-erbB-2, EGFR and ras were examined on the adjacent sections of 75 cases of gastric cancer (GC), and their clinicopathological and prognostic significance were studied. Results: 1) Overexpressions of p53 and c-erbB-2 were early and late sign of malignant change of gastric mucosa, respectively; EGFR and ras had close association with the degree of malignancy of GC. 2) The expression of ras had positive relationship with the expression of EGFR, and negative relationship with c-erbB-2.3). The multivariate analysis of Cox model of GC showed only DNA ploidy, expression of EGFR, lymph node metastasis and distant metastasis were independent prognostic factors of GC.

Key Words: Stomach neoplasms, Oncogene, Immunohistochemistry, Gene expression

Activation of cellular oncogenes and inactivation of tumor suppresser genes are in the course of gastrocarcinomagenesis and maintance of malignant phenotype. Now not a few researchers have been trying to study the clinic significance of genetic abnormalities in gastric cancer.[1-3] But many of them studied with univariate analysis, so the results reported are usually controversial and not reliable. In this study the immunohistochemical expressions of tumor suppresser gene p53 and several oncogenes (EGFR[Epidermal Growth Factor Receptor], ras, and c-erbB-2) were studied.

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MATERIALS AND METHODS

Tissue Materials

Tissues from 75 cases of advanced gastric cancer over an 5-year period (1986-1991) were obtained from the Xihua Hospital, Shanghai. They were routinely fixed in 10% formalin and paraffin-embedded. Adjacent sections were cut at 5 μm and mounted on poly-l-lysine-coated glass slides. ras, EGFR, c-erbB-2 and p53 were examined on ajacent sections to H.E. staining section.

Main Reagents Used and Immunohistochemistry

For ras, EGFR and p53 detections, the labeled streptoavidinebiotinperoxidase complex (LSAB) technique was used. The final antibody concentrations of p53 (DO-7, Novacastra), EGFR (Sigma) and ras (Pan-ras, Oncogene Sci) were 1: 20; 1: 700 and 1: 5, respectively. For c-erbB-2 detection a rabbit polyclonal antibody to c-erbB-2 (Dako) was used at a final concentration of 1: 100. The avidin-biotin-peroxidasecomplex (ABC) technique was used to demonstrate c-erbB-2 immunoreactivity. The subsequent steps were performed according to the manufacturer's instructions. Breast cancer and colon carcinoma were used as positive controls. Negative controls for the immunostaining were carried out by replacing primary antibodies with normal mouse or rabbit serum. Each section was evaluated according to the intensity of immunoreaction and the percentage of positive cells: −, no staining; +, faint or focal (<50%

of tumor); ++, strong or diffuse (>50% of tumor).

Statistical Analysis

X^2 test was used to analysis the relationships between expressions of ras, c-erbB-2, p53 and EGFR and the clinicopathological features of gastric cancer.

RESULTS

Expressions of p53, c-erbB-2, EGFR and ras

The frequencies of expressions of p53, c-erbB- 2, EGFR and ras in cancer site were 41.3% (31/75), 1.7% (14/75), 61.3% (46/75), respectively. All positive immunoreactivity of p53 was in cell nuclear. For c-erbB-2, EGFR and ras mainly on cell memberance, and a few in cytoplasm. Except for two cases of severe dysplasia lesions, expression of p53 in all other tissues adjacent to cancer was negative, including 20 cases of mild dysplasia, 26 cases of moderate dysplasia, 30 cases of metaplasia and normal mucosa epethelial cells. c-erbB-2 positive immunoreactivity was only localized in the cancer cells. But EGFR and ras staining could be found in the tissues adjacent to the cancer cells. Relationship between multiple genetic expressions and clinicopathological features of gastric cancer was demonstrated in Table 1. Relationship between expression of EGFR and c-erbB-2: From Table 2 we can see there were no significant relationship (P >0.05). Relationship between multiple genetic expressions could be found in Table 3.

Table 1. Relationship between multiple genetic expressions and clinicopathological features of gastric cancer

Features	cases	Positive (%)			
		p53	c-erbB-2	EGFR	ras
Sex					
male	58	24 (41.4)	9 (15.5)	35 (60.3)	25 (43.1)
female	17	7 (41.2)	5 (29.4)	11 (64.7)	10 (58.8)
Age (years)					
<60	35	13 (37.1)	4 (11.0)	24 (68.6)	18 (45.7)
>60	40	18 (45.0)	10 (25.0)	22 (55.0)	17 (42.5)
Location					
C	18	7 (38.9)	5 (27.8)	10 (55.6)	7 (38.8)
M	12	3 (25.0)	2 (16.7)	6 (50.0)	7 (58.3)
A	45	21 (46.7)	7 (15.6)	30 (66.7)	21 (46.7
Borrman's type[V]					
gross localized	25	12 (48.0)	9 (36.0)	11 (44.0)*	9 (36.0)
gross infiltrative	40	13 (32.5)	4 (10.0)	29 (72.5)	22 (55.0)
Lauren's type					
intestinal	38	20 (52.6)*	10 (26.3)	20 (52.6)	15 (39.5)
diffuse	37	11 (29.7)	4 (10.8)	26 (70.3)	20 (54.1)
Growth pattern					
expansive	29	15 (51.7)	8 (27.5)	13 (44.8)	8 (27.9)
infiltrative	46	16 (34.8)	6 (13.0)	33 (71.7)	27 (58.7)
Differentiation[VV]					
well	38	18 (47.4)	11 (28.9)*	19 (50.0)	11 (28.9)
poor	37	13 (35.1)	3 (8. 1)	27 (73.0)	24 (64.9)
Depth of invasion					
within serosine	39	19 (48.7)	10 (25.6)	22 (56.4)	10 (25.6)
out of serosine	36	12 (33.3)	4 (11.1)	24 (66.7)	25 (64.9)
Lymph metastasis					
No	30	13 (43.3)	3 (10.0)	14 (46.7)	7 (23.3)
Yes	45	18 (40.0)	11 (24.4)	32 (71.1)	28 (62.2)

Stage					
early	10	6 (60.0)	1 (10.0)	6 (60.0)	4 (40.0)
advance	65	25 (38.5)	13 (20.0)	40 (61.5)	31 (47.7)
Distant metastasis					
No	68	28 (41.2)	13 (19.1)	39 (57.4)*	32 (47.1)
Yes	7	3 (42.9)	1 (14.3)	7 (100)	3 (42.8)
TNM stage					
1+ 2a	27	12 (44.4)	3 (11.1)	15 (55.6)	9 (33.3)
2b + 3a	28	14 (50.0)	8 (28.6)	18 (64.3)	13 (46.4)
3b + 4	20	5 (25.0)	3 (15.0)	13 (65.0)	13 (65.0)

* $P<0.05$ ^{V} gross localized type: Borrman 1+2; gross infiltrative type: Borrman 3+4; ^{VV} well differentiation: grade 1+2; the poor: grade 3+4.

Table 2. Relationship between expressions of c-erbB-2 and EGFR

EGFR	cases (n)	c-erbB-2			
		Negative		positive	
		cases (n)	%	cases (n)	%
positive	29	23	79.3	6	20.7
negative	46	38	82.6	8	17.4

Note: EGFR vs c-erbB-2 $P>0.05$

Table 3. Relationship between multiple genetic expressions

	Cases(n)	c-erbB-2		EGFR	
		positive	%	positive	%
ras					
negative	40	11	27. 5*	19	47. 5**
positive	35	3	8. 6	27	77. 1
p53					
negative	44	9	20. 5	28	63. 6
positive	41	5	16. 1	18	58. 1

Note: *$P<0.05$, **$P<0.01$

DISCUSSION

Recent study has shown that, in the course of gastrocarcinomagenesis and progression, several genetic abnormalities are involved, including the activation of proto-oncogenes and inactivation of tumor suppresser genes. By means of immunohistochemistry, the expression products of these genetic abnormalities could be examined. Expressions of multiple genetic abnormalities on adjacent sectionscan provide location comparison.

p53 is a tumor suppresser gene coding for a phospholipid protein product. This study showed more expression of p53 in gastric cancer of expansive growth, well-differentiation, and there was no relationship between p53 expression and parameters of invasion and metastasis of gastric cancer. So it seems that p53 expression hasn't prognostic significance. Rugge, et al.[4] showed that there was p53 staining in severe and moderate dysplasia tissue. Besides, Uchino, et al.[5] proved that there was p53 gene mutation in 53.3 percent of metaplasia tissue with PCR-SSCP and DNA sequence method. So we think p53 expression could be an early event in the course of gastrocarcinomagenesis and plays a weak role in the course of progression of gastric cancer. c-erbB-2

oncogene activation is related to progression in tumors of hormonally sensitive tissues, such as those of breast and ovary.[6,7] It has been studied in other tumors, including gastric cancer. In our series c-erbB-2 immunoreactivity was located only in cancer cells and no in precarcinoma tissues. This indicate that overexpression of c-erbB-2 may be a late event of carcinoma transformation. Our study showed that there was no association between c-erbB-2 expression and parameters related to malignant potentiality of gastric cancer. So we think c-erbB-2 protein in gastric cancer may have no prognostic significance. We found more expressions of c-erbB-2 and p53 in intestinal type cases of gastric cancer than in diffuse type ones. It may indicate that there is difference between these two types not only in features of biological behavior and epidemiology but also in molecular machinism of carcinomagenesis.[4,9]

In the present study EGFR-positive gastric carcinomas showed EGFR, a 170-kD glycoprotein is a transmembrane tyrosine kinase. The binding of the ligand EGF and or its hemologous protein TGF to EGFR activates a signal transduction system and stimulates cellular growth and proliferation. So more expression of EGFR could be seen in more malignant tumor.[1,9] It contains three domains: an external ligand-binding domain, a transmembrance domain, and an internal domain that has tyrosine kinase activity.

ras oncogene plays a role in cell growth and differentiation.[10] The p21 protein encoded by the ras gene family functions as a g protein that participates in membrane signal transduction pathways.[12,13]

The positive association between expressions of EGFR and ras further proved that the two factors plays a significant role in the progression of gastric cancer. And the negative relationship between c-erbB-2 and ras showed the different role in the gastrocarcinoma-genesis and progression from ras and EGFR.

In conclusion, several genetic abnormalities are involved in the gastrocarcinomagenesis. Multiple genetic examination has diagnostic and prognostic significance.

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