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ARTICLE

The Expression of c-erbB-1 and c-erbB-2 in Iranian Patients with Gastric Carcinoma

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To assess the significance of epidermal growth factor receptor family members, the overexpression of c-erbB-1 and c-erbB-2 was retrospectively investigated in 146 southern Iranian gastric cancer patients. Indirect immunostaining was used to evaluate the expression of these two receptors in formalin-fixed paraffin-embedded tissue samples. c-ErbB-1 expression was observed in 47 (32.2%) and c-erbB-2 expression was observed in 24 (16.4%) of tumors. Significant positive correlations were observed between c-erbB-1 expression and tumor size, local invasion, lymph node involvement and tumor stage. There was also a negative correlation between c-erbB-2 expression and tumor stage. These results may suggest the contribution of c-erbB-1 molecule in progression of gastric carcinomas in southern Iranian patients. Moreover, the relatively high percentage of c-erbB-2 positive tumors may provide a useful target for the immunotherapy of these cancers. (Pathology Oncology Research Vol 8, No 4, 252–256)

Keywords: c-erbB-1, c-erbB-2, gastric carcinoma, southern Iranian

Introduction

Gastric cancer is the third most prevalent cancer in Fars, Iran, ranked after skin and breast cancers.[1] The total number of registered gastric cancer cases in year 1999 amounted to 201 which is 9.4% of total registered cancer cases in the same period.[1] The general prognosis of gastric tumors is poor and patients respond poorly to the conventional treatments. Although a high degree of inconsistency exists between different studies on the important prognostic factors in gastric cancer, in most studies a high level of expression of epidermal growth factor receptor family members is reported.[2,3,4,5,6,7] Epidermal growth factor receptor (EGFR/HERA/c-erbB1) and c-erbB-2 expression in gastric carcinoma correlates with different clinicopathological characteristics of tumors including depth of penetration, metastatic behavior and stage of tumor.[2,3,8,9] Collectively, these molecules are considered as poor prognostic factors in gastric carcinoma.[9,10,11,12]

These data have resulted to the consideration of c-erbB-1 and/or c-erbB-2 as a target of therapy in gastric cancer and other tumors of gastrointestinal tract.[13,14,15] Accordingly, several anti-human c-erbB-1 monoclonal antibodies have been produced and studied for the treatment of different cancers,[16,17,18] among which a chimeric human-mouse monoclonal antibody against this molecule has been shown to have both *in vitro* and *in vivo* antitumor activity against colon cancer cells, when used alone or in combination with other anti-cancer procedures.[17,18,19] This antibody has been used in phase II trials in patients with head and neck and lung carcinomas.[20] Moreover, a recently approved humanized monoclonal antibody against c-erbB-2 molecule has provided intriguing results in the treatment of breast cancer either alone[21] or in combination with chemotherapy.[22] No major side effects have been reported except the cardiotoxicity when it is used in combination with anthracyclines.[23,24] The use of this approach in Non-Small Cell Lung Cancer (NSCLC) has also been

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notified by some investigators.[25] Preliminary data from a phase II clinical trial of this approach in patients with NSCLC has revealed a 21-40% response rate.[26]

In the treatment of breast cancer patients, it has been shown that patients who overexpress c-erbB-2 molecule in their tumors, benefit more from anti-c-erbB-2 therapy[21] and this is more obvious when the immunohistochemical staining is at the highest levels.[27] The same correlation between c-erbB-2 overexpression and benefit from antibody treatment is reported in NSCLC patients.[26]

Due to the availability of immunotherapy approaches for Iranian breast cancer patients, a question was raised about the number and percentage of Iranian gastric cancer patients who overexpress c-erbB-1 and c-erbB-2 and therefore may benefit from the possible future immunotherapy. To answer this question and as the first study on the overexpression of epidermal growth factor receptors in Iranian gastric cancer patients, this study was undertaken.

Materials and methods

Tumors

146 formalin fixed specimens of gastric carcinoma patients who were operated at Nemazi and Faghihi hospitals (University hospitals, affiliated to Shiraz University of Medical Sciences, Shiraz, Iran) between 1996 and 2000 formed the study material for this project. Clinocopathological data were recorded and specimens from known cases were used as positive controls for immunohistochmical analysis.

Antibodies and conjugates

For detection of c-erbB1 and c-erbB-2 molecules, ICR16[28] and ICR12[29] rat monoclonal antibodies were used, respectively. Sheep anti-rat/HRP antibody (Amersham Co, U.K.) was used as the secondary antibody in detection of these receptors.

Immunohistochemistry

An indirect immunoperoxidase method was used for detection of c-erbB1 and c-erbB-2 on formalin-fixed paraffin-embedded tissue sections. Multiple 5 μm sections were cut and stored at 4°C until immunohitochemical study. For detection of c-erbB1 and c-erbB-2 molecules the following procedure was carried out: After deparaffinization by using xylene and rehydration in graded ethanol solutions, sections were washed with phosphate buffered saline (PBS). Endogenous peroxidase was blocked by immersing the sections in PBS containing 3% H_2O_2 for 15 minutes. The sections were then incubated with 1/100 dilution of primary antibodies (ICR16 or ICR12) overnight in 4°C. the slides were washed three times in PBS containing 0.05% Tween 20 and then incubated with 1/100 dilution of sheep conjugated anti-rat antibody (Amersham Co, U.K.) for 90 minutes at room temperature. After further washing in PBS-Tween 20 (0.05%) the final reaction product was visualized using diaminobenzidine (DAB) [100 mg DAB in 100 ml of PBS (pH 7.2), 100 ml H_2O, 66 μl H_2O_2]. After 5 minutes, the sections were washed twice in distilled water and counter-stained in hematoxyline and mounted with Entellan. After dehydration and mounting, the slides were examined visually by a light microscope. Membrane or cytoplasmic staining was considered as positive for detection of c-erbB-1 and c-erbB-2. In each experiment negative controls for which the first antibody was substituted with PBS were included. Two c-erbB-1 and c-erbB-2 positive breast adenocarcinomas were used as positive controls for immunostaining.

Table 1. **Characteristics of tumors**

	No. of cases
Tumor size (cm)	
< 6	68
> 6	78
Location	
Cardia	49
Body	36
Antrum	61
Local invasion	
Negative	57
Positive	89
Distant metastasis	
Negative	16
Positive	130
Histology	
Intestinal	96
Diffuse	42
Early cancer	8
Nodal involvement	
Negative	67
Positive	79
Serosal involvement	
Positive	108
Negative	38
Stage	
I	9
II	31
III	92
IV	14
Nuclear grade	
1	35
2	38
3	73

Statistical analysis

Microsoft EPI-6 was used for statistical analysis. P values were calculated by using Chi-square or Fisher exact tests.

Results

Clinicopathological data

Of the 146 gastric cancer patients, 127 were male and 19 were female. Median age was 66 ranged between 33-79 years. As it is shown in *Table 1*, 68 (46.6%) samples were from tumors smaller than 6 cm and 78 (53.4%) were from tumors equal or larger than 6 cm in size. A high percentage (72.6%) of tumors was in stages III or IV and nodal involvement was observed in 79 (54.2%) tumors. In addition, local invasion and distant metastasis were observed in 89 (61%) and 130 (89%) cases, respectively.

Association between c-erbB-1 expression and clinicopathological parameters

In 47 out of 146 (32.2%) of samples c-erbB-1 expression was observed and in most cases the reaction was localized to both cell membrane and cytoplasm. Positive reactivity with anti-c-erbB-1 antibody was more frequently observed in larger tumors (diameter greater than 6 cm) than smaller tumors (diameter less than 6 cm) (41% Vs 22%, P = 0.023). Nine out of 57 (15.8%) of the tumors with no local invasion were positive for c-erbB-1, however, 38 out of 89 (42.7%) of tumors with local invasion were positive for this receptor. A significant difference in the expression of c-erbB-1 in tumors with local invasion and those without local invasion was observed (P = 0.001). There was also a significant difference in the c-erbB-1 expression between node negative and node positive tumors (P = 0.031). 32 out of 79 (40.5%) cases with node positive and 15 out of 67 (22.4%) cases with node negative were positive for c-erbB-1 receptor. According to the TNM tumor staging, a significant correlation between stage of the tumors and c-erbB-1 expression was observed (P = 0.0002) (*Table 2*). In this regard, high stage tumors (III/IV) were more likely to overexpress c-erbB-1 molecule.

Association between c-erbB-2 expression and clinicopathological parameters

Positive staining for this receptor was observed in 24 out of 146 (16.4%) of tumors. As in case of c-erbB-1, the immunoreactivity was localized in both cell membrane and cytoplasm, however, the intensity of staining were stonger than c-erbB-1. 11 out of 40 (27.5%) of low stage tumors (I/II) and 13 out of 106 (12.3%) of high stage (III/IV) tumors showed positive staining for c-erbB-2, and this difference was statistically significant (P = 0.049). With the exception of tumor stage, no other correlation was observed between the overexpression of this molecule and the studied variabels (*Table 3*).

Discussion

In the present study we investigated the expression of two members of the epidemal growth factor receptor family in 146 southern Iranian gastric cancer patients, retrospectively. Epidermal growth factor receptor family comprises of four members with similar basic structure containing an intracytoplasmic tyrosine kinase domain.[30] These receptors play important roles in proliferation and differentiation of normal cells. Consequently, any abberration in their structure or function may contribute in the cancer development or progression.[31] c-erbB-1 and

Table 2. **The expression of c-erbB-1 in patients with gastric carcinoma**

	c-erbB-1 staining		
	Negative	*Positive*	*P value*
Tumor size (cm)			0.023
< 6	53	15	
≥ 6	46	32	
Location			N.S.
Cardia	36	13	
Body	22	14	
Antrum	41	20	
Local invasion			0.001
Negative	48	9	
Positive	51	38	
Distant metastasis			N.S.
Negative	13	3	
Positive	86	44	
Histology			N.S.
Intestinal	61	35	
Diffuse	30	12	
Early cancer	8	0	
Nodal involvement			0.031
Negative	52	15	
Positive	47	32	
Serosal involvement			N.S
Negative	28	10	
Positive	71	37	
Stage			0.0002
I / II	37	3	
III / IV	62	44	
Nuclear grade			N.S.
1	22	13	
2	24	14	
3	53	20	

Table 3. **The expression of c-erbB-2 in patients with gastric carcinoma**

	c-erbB-2 staining		
	Negative	*Positive*	*P value*
Tumor size (cm)			N.S
< 6	54	14	
≥ 6	68	10	
Location			N.S.
Cardia	41	8	
Body	29	7	
Antrum	52	9	
Local invasion			N.S.
Negative	48	9	
Positive	74	15	
Distant metastasis			N.S.
Negative	12	4	
Positive	110	20	
Histology			N.S.
Intestinal	83	18	
Diffuse	33	5	
Early cancer	7	1	
Nodal involvement			N.S.
Negative	60	7	
Positive	62	17	
Serosal involvement			N.S.
Negative*	33	4	
Positive	88	20	
Stage			0.049
I / II	29	11	
III / IV	93	13	
Nuclear grade			N.S.
1	26	9	
2	32	6	
3	64	9	

* One case was missing

c-erbB-2 proteins differ in size and also are encoded by genes located on chromosomes 7 and 17, respectively. The overexpression of these two molecules in gastric cancer and its correlation with the outcome of the disease has been a subject of interest over the last decade. Different rates of overexpression of c-erbB-1 ranging from 7.1% to 80% have been reported in gastric tumors.[2,11] Similarly the range of overexpression of c-erbB-2 varies from 8.2% to 62.5% in different reports.[11,32] In our study, c-erbB-1 and c-erbB-2 were found to be overexpressed in 32.2% and 16.4% of gatric tumor samples. In addition, c-erbB-1 expression revealed a positive correlation with the size of tumor, local invasion of tumor and lymph node involvement. These results are in agreement with the previous reports on the correlation of metastatic behavior and invasiveness of gastric tumors with c-erbB-1 expression.[2,3,10] We also observed a positive correlation between stage of the tumors and c-erbB-1 expression. These results suggest the contribution of the c-erbB-1 molecule in the progression and invasion of gastric tumors in Iranian patients and can be considered as a prognostic marker in these tumors. However, this is contrary to the breast cancer and head and neck tumors in southern Iran in which the overexpression of this molecule has no correlation with the clinicopathological data.[33,34] The same higher expression of c-erbB-1 in poorly differentiated gastric tumors has been reported by Prakash et al[3] in 1997. The expression of c-erbB-2 molecule negatively correlated with the tumor stage, i.e. well differentiated tumors were more likely to overexpress the c-erbB-2 molecule. This is in agreement with the results reported by other investigators.[9,35]

The newly developed immunotherapy approaches for the treatment of cancer has made c-erbB-1 and c-erbB-2 molecules as useful targets for treatment of human tumors. Therefore, the possible implications of anti-c-erbB1 and anti-c-erbB2 molecules in the treatment of Iranian gastric cancer patients should be considered. In addition, the overexpression of c-erbB-1 and its positive correlation with prognostic factors in our patients may provide a useful tool for diagnosis and prognosis of Iranian gastric patients. It is therefore suggested that the screening of these patients for expression of this molecule be routinely performed.

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