Clinical Significance of Hyperexpression of Epidermal Growth Factor Receptors (EGFR and HER-2) in Esophageal Squamous Cell Carcinoma

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Immunohistochemical study of marker expression in primary tumors of patients with esophageal squamous-cell carcinoma was carried out in order to evaluate prognostic significance of EGFR and HER-2 levels in the tumors. Hyperexpression of EGFR and HER-2 in the tumors is an important marker for the analysis of prognosis and clinical course of the disease. A relationship between high levels of EGFR and HER-2 in the tumors of patients with esophageal squamous-cell carcinoma and intravascular tumor invasion (p=0.038) and poor outcome of the disease (p=0.019) was detected. The results indicate that evaluation of changes in the expression of EGFR and HER-2 in tumors is essential for individual prediction of the disease course and development of new approaches to the treatment of these tumors, including target therapy aimed at these tyrosine kinase receptors.

Key Words: *esophageal cancer; immunohistochemistry; EGFR; HER-2; prognosis*

Esophageal squamous-cell carcinoma (ESCC) by its clinical course is one of the most aggressive malignant tumors in humans. Its unfavorable prognosis is largely explained by biological characteristics of the tumor. The study of clinical significance of molecular biological parameters predicting the risk of disease progress is explained by the need to detect the risk groups among ESCC patients for prescribing more effective therapy. The malignant phenotype of the tumor is determined by disorders in the expression of molecular factors essential for tumor cell growth and proliferative activity, for example, growth factors and their receptors [7,8,12].

The EGFR (HER-1, ERBB-1,) and HER-2 (ERBB-2) are representatives of the family of the epidermal growth factor transmembrane receptors with tyrosine kinase activity (molecular weights 170 and 185 kD, respectively). The receptors are involved in the development of a series of human malignant tumors and are now actively studied as therapeutic antitumor targets [2,5,10,12]. Stimulation of the receptors triggers the transcription mechanisms accelerating the proliferation of epithelial cells. In addition, these molecules regulate tumor invasion and neoangiogenesis processes by autocrine and/or paracrine metabolic mechanisms [12,15]. It was found that hyperexpression of EGFR and HER-2 on tumor cell membrane is linked with amplification of the respective genes and correlates with unfavorable prognosis and aggressive course of human malignant tumors [15].

Expression of EGFR is detected in 50-80% patients with ESCC [4,10], the incidence of HER-2 hyperexpression varies from 0 to 56% [9,12]. Studies of the prognostic significance of abnormal expression of EGFR and HER-2 in ESCC have recently led some authors to a hypothesis according to which hyperexpression of these receptors in the tumor correlates with unfavorable postoperative prognosis of the disease course [1,2,3,14]. In addition, HER-2 hyperexpression

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is associated with deterioration of overall survival of ESCC patients [2,14], while EGFR hyperexpression correlates with deeper invasion of the tumor, intravascular invasion, and risk of local relapses [3,6]. In many cases, a clear-cut correlation between EGFR and HER-2 genes amplification (detected by FISH reaction) and hyperexpression of the respective proteins (detected by immunohistochemical studies) is observed in ESCC [6,11,13,14]. However, the results of studies of the relationship between HER-2 gene amplification or protein hyperexpression and disease prognosis remain contradictory.

We studied the status of epidermal growth factor receptors (EGFR and HER-2) in the tumors of ESCC patients and evaluated the relationship between their content in the tumor and the main clinical morphological characteristics and disease prognosis.

MATERIALS AND METHODS

The expression of EGFR and HER-2 was studied in postoperative specimens of tumors from 28 patients with stages II-III esophageal cancer (6 women and 22 men aged 44-72 years). Eighteen (64.3%) patients had metastases in regional lymph nodes before operation. Sixteen (57.1%) patients developed local relapses and/or distant metastases during 1-3 years. Twelve patients lived for 3 years and longer without disease progress. No preoperative chemoradiotherapy was carried out in this group of ESCC patients.

Histological study of the material showed that all esophageal tumors were squamous-cell carcinomas with hornification of different degree. Well-differentiated cancer (G1) was diagnosed in 2 (7.1%) patients, moderately differentiated (G2) in 19 (67.9%), and

TABLE 1. Clinical Morphological Parameters and Prognostic Values

Sign		Number of cases	
		abs.	%
Sex	m	22	77.8
	f	6	22.2
Differentiation degree	G1 high	2	7.1
	G2 moderate	19	67.9
	G3 poor	7	25.0
Depth of invasion	Submucosa	1	3.6
	Muscular layer	10	35.7
	Adventitia	9	32.1
	Fat	8	28.6
Intravascular invasion	None	7	25.0
	Present	21	75.0
pT	1	1	3.6
	2	11	39.3
	3	10	35.7
	4	6	21.4
pN	0	11	39.3
	1	17	60.7
Stage	IIA	9	32.1
	IIB	5	17.9
	III	14	50.0
Development of local relapse or distant metastases	Yes	12	42.9
	No	16	57.1

Note. Here and in Tables 2 and 3: *n*: number of patients.

poorly differentiated (G3) in 7 (25%) patients. The following clinical parameters were taken into consideration: depth of tumor invasion in esophageal wall and intravascular invasion and the presence of lymphoid infiltration in the tumor, desmoplastic reaction of the stroma, perineural invasion. Clinical morphological characteristics of the material are summed up in Table 1.

Immunohistochemical staining was carried out on paraffin sections of esophageal tumors using antibodies to EGFR (Novocastra) and Super Sensitive Polymer-HRP detection system (BioGenex). The expression of HER-2 was evaluated by HercepTest system (Dako). The antigenic structure was restored by treatment of deparaffinized sections in citrate buffer (pH 6.0) in a water bath at 95°C for 40 min. The results were evaluated by HercepTest system from "0" to "3+". The intensity of staining of tumor cell membranes was evaluated.

Mathematical analysis of the results was carried out using SPSS 17.0 and Excel software. The significance of differences in the frequencies of signs in the studied groups was evaluated using nonparametric exact Fisher test for small samples (the differences were considered significant at $p<0.05$).

RESULTS

Study of EGFR expression showed positive immunoreactivity of tumor cell membranes in the majority of tumors. Negative reaction was recorded in only 4 (14.3%) cases: poor expression (1+) in 3 (10.7%) cases and complete absence of immunoreactivity (0) in 1 (3.6%) case (Fig. 1, *a*). Positive expression of EGFR in ESCC cells was observed in 24 (85.7%) cases: moderate immunoreactivity (2+) in 11 (39.3%) cases (Fig. 1, *b*) and hyperexpression (3+) in 13 (46.4%) cases (Fig. 1, *c*). Positive reaction presented by diffuse homogenous staining of complexes and cords of squamous-cell carcinoma or was located at the periphery in the basal compartments of tumor growth. Intensive diffuse staining of the entire membrane of the majority of tumor cells (2+/3+) was associated with intravascular invasion in the tumor ($p=0.038$) and was more often observed in the subgroup of patients who developed local relapses or metastases early after surgery. However, the differences in the disease outcomes in ESCC patients with EGFR-positive and EGFR-negative tumors were not significant (Table 2).

Analysis of HER-2 expression in the studied ESCC cases showed differences in the staining intensity, distribution and quantity of antigen-positive cells in the thickness of tumor cords. Immunoreactivity of cell membranes was diffuse at the periphery and in central compartments of tumor growth in the majority of cases. In some ESCC samples, the intensity of membrane expression of HER-2 increased towards the basal compartments of epithelial cords and complexes and was maximum in small groups of separated tumor cells.

Positive expression of HER-2 in ESCC cells was observed in 17 (60.8%) cases: moderate immunoreactivity (2+) in 12 (42.9%) and hyperexpression (3+) in 5 (17.9%) cases (Fig. 1, *d*). Negative immunoreactivity was detected in 11 (39.3%) cases: poor expression with uneven staining of membrane surface of solitary tumor cells (1+) in 6 (21.4%) cases and complete absence of immunoreactivity (0) in 5 (17.9%) cases.

Moderate expression of both receptors mainly in the basal layers of the epithelium was seen in the esophageal mucosal areas adjacent to the tumor. In some cases hyperplasia and slight or moderate dysplasia of esophageal epithelium were observed; the cell staining in the thickness of the epithelial layer was more intense in these cases.

Hyperexpression of HER-2 in the tumor did not correlate with any clinical morphological parameters of the disease. The level of the receptor expression

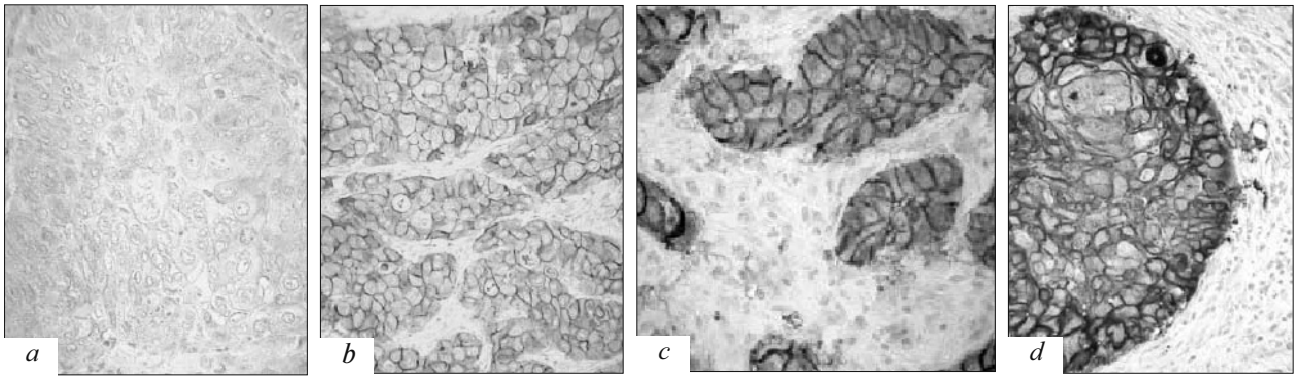

Fig. 1. Immunohistochemical detection of membrane expression of EGFR and HER-2 in ESCC cells. *a*) EGFR-negative expression, ×250; *b*) EGFR-positive expression (2+), ×250; *c*) EGFR-positive expression (+3), ×400; *d*) HER-2-positive expression (3+), ×400. Cell nuclei poststained with Meyer's hematoxylin.

TABLE 2. Relationship between EGFR Expression in ESCC and Clinical Morphological Parameters of the Disease (Analysis of Subgroups)

Parameter		EGFR		Total, *n*	*p*
		negative, 0/1+, % (*n*=4)	positive, 2+/3+, % (*n*=24)		
Differentiation degree	G1 and G2	3 (10.7)	18 (64.3)	21	1.000
	G3	1 (3.6)	6 (21.4)	7	
Depth of invasion	P1 and P2	1 (3.6)	10 (35.7)	11	1.000
	P3 and P4	3 (10.7)	14 (50.0)	17	
Vascular invasion	No	3 (10.7)	4 (14.3)	7	0.038*
	Yes	1 (3.6)	20 (71.4)	21	
Lymphoid infiltration	No	1 (3.6)	8 (7.1)	9	1.000
	Yes	3 (10.7)	16 (57.1)	19	
pN	0	2 (7.1)	8 (7.1)	10	0.135
	1	2 (7.1)	16 (57.1)	18	
pT	1 and 2	2 (7.1)	10 (35.7)	12	1.000
	3 and 4	2 (7.1)	14 (50.0)	16	
Development of relapse and metastases	No	3 (10.7)	9 (32.1)	12	0.285
	Yes	1 (3.6)	15 (53.6)	16	

Note. Here and in Table 3: *statistically significant difference ($p<0.05$). The percent age is shown in parentheses.

TABLE 3. Relationship between HER-2 Expression in ESCC and Clinical Morphological Parameters (Analysis of Subgroups)

Parameter		HER-2		Total, *n*	*p*
		negative, 0/1+, % (*n*=11)	positive, 2+/3+, % (*n*=17)		
Differentiation degree	G1 and G2	9 (3.6)	12 (3.6)	21	0.668
	G3	2 (7.1)	5 (17.9)	7	
Depth of invasion	P1 and P2	2 (7.1)	9 (32.1)	11	0.115
	P3 and P4	9 (32.1)	8 (28.6)	17	
Vascular invasion	No	4 (14.3)	3 (10.7)	7	0.381
	Yes	7 (25.0)	14 (50.0)	21	
Lymphoid infiltration	No	5 (17.9)	4 (14.3)	9	0.409
	Yes	6 (21.4)	13 (46.4)	19	
pN	0	3 (10.7)	7 (25.0)	10	0.689
	1	8 (28.6)	10 (35.7)	18	
pT	1 and 2	2 (7.1)	10 (35.7)	12	1.000
	3 and 4	9 (32.4)	7 (25.0)	16	
Development of relapse and metastases	No	8 (28.6)	4 (14.3)	12	0.019*
	Yes	3 (10.7)	13 (46.4)	16	

was higher in the presence of intravascular invasion and deeper invasion of the tumor into esophageal wall, but the differences were insignificant (Table 3).

Membrane hyperexpression of HER-2 in tumor cells was an indicator of unfavorable outcome and was more often detected in the patients who died early after surgery from the disease progress than in patients surviving for more than 3 years without distant metastases and local relapses (p=0.019; Table 3).

Hyperexpression of HER-2 in ESCC was associated with high expression of EGFR in cell membranes in 4 (14.3%) tumors of 28; diffuse location or basal distribution of both markers were observed in these cases.

Hence, ESCC is characterized by pronounced expression of EGFR and HER-2 in tumor cell membranes in epithelial complexes and cords. A relationship between high level of EGFR in ESCC cells and intravascular tumor invasion was detected (p=0.038). Hyperexpression of HER-2 in the tumor was associated with ESCC progress and was significantly more incident in patients developing local relapses or distant metastases early after surgery (p=0.019). In addition, evaluation of EGFR and HER-2 expression in the tumors of ESCC patients can be used for individual prediction of the clinical course of disease and response to chemotherapy, as well as for the development of new therapeutic protocols of target therapy aimed at these receptors.

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