Exp Oncol 2008
30, 3, 235–239

Experimental ONCOLOGY

RELATION BETWEEN CELL-TO-CELL ADHESION AND ANGIOGENESIS AND CLINICO-MORPHOLOGICAL PROGNOSTIC FACTORS IN PATIENTS WITH GASTRIC CANCER

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Aim: **To study the relation between the expression of the molecules of cell-to-cell adhesion (E-cadherin, α- and β-catenins) and vascular endothelial growth factor (VEGF) and traditional clinico-morphological characteristics of tumors to evaluate their prognostic value in the patients with gastric cancer.** ***Methods:*** **To analyze the expression of E-cadherin, α- and β-catenins, and VEGF the paraffin embedded tumor samples were studied by immunohistochemical analysis with the use of respective monoclonal antibodies.** ***Results:*** **The presence of E-cadherin in tumors correlated with the absence of metastases in regional lymph nodes and was observed, as a rule, in the patients at the early stages of the disease. The presence of β-catenin expression has been detected in gastric tumors of the patients without distant metastases, while the level of VEGF expression correlated with the degree of gastric wall injury. It has been demonstrated that the expression of E-cadherin and α-catenin is associated with favourable disease course and is a characteristic pattern for early stages of gastric cancer of intestinal type. However, VEGF expression is typical for the late stages of gastric cancer of diffuse type and is associated with poor prognosis.** ***Conclusion:*** **At the base of combined clinical, histological and immunohistochemical analysis of gastric tumors it has been shown that E-cadherin, α-catenin and VEGF could be used as informative markers of the disease course.**
Key Words: **gastric cancer, prognosis, E-cadherin, alpha-catenin, beta-catenin, VEGF.**

According to WHO data, gastric cancer (GC) occupies the second place by the incidence and mortality among all malignant neoplasms. GC morbidity yields 880 000 cases per year, while mortality rate is close to 650,000 per year [1].

High mortality rate largely depends on the fact that GC is usually revealed at the late stages (III–IV) of the disease [2, 3]. At the same time, the results of the therapy differ in a wide range in the GC patients with the tumors of similar histology and progression stage. Due to the advances of modern genetics and molecular biology, it became evident that molecular-biological properties of the tumor play a key role in the GC prognosis [4].

According to the data of a number of researchers [5–13], disturbed expression of the components of cell-to-cell adhesion (cadherin-catenin complex: E-cadherin, α- and β-catenin) is an important event in the development and progression of GC. An absence of expression of each separate protein forming the complex is associated with unfavorable disease course, and appearance of regional or distant metastases [14, 15]. It is known also that in some cancers the aggressive course of the disease correlates with altered level of expression of growth factors stimulating angiogenesis [16]. Vascular endothelial growth factor (VEGF) is a key regulator of angiogenesis and induces proliferation of endothelium along with the formation of new vessels of capillary type. At the same time, apart from proangiogenic activity, VEGF also influences mitogenic (Akt) and antiapoptotic (BCL-2) proteins [17]. There is no common point of view in modern literature on the prognostic value of expression level of the components of cell-to-cell adhesion system and the markers of angiogenesis in gastric cancer.

The aim of the present research was to study the relation between expression of the molecules of cell-to-cell adhesion (E-cadherin, α- and β-catenin) and VEGF with traditional clinicomorphological characteristics of tumors to evaluate their prognostic value in GC patients.

MATERIALS AND METHODS

Retrospective study of biological parameters of tumors of 150 GC patients cured in State Institution "National Cancer Institute" Ministry of Health of Ukraine (Kyiv, Ukraine) in 1998–2004. All patients provided an informed written concent to perform the study, and the present research was approved by Ethic Committee of the Institute. The data on patients' gender, stage of the disease, histological structure and differentiation grade of tumors are presented in Table 1.

Table 1. Clinico-morphological characteristics of the patients with gastric cancer

Parameters		Number of samples (n)
Gender	Male	89
	Female	61
Stage of the disease by TNM	II	30
	III	54
	IV	57
"T"	T1	1
	T2	11
	T3	80
	T4	58
"N"	N0	63
	N1	45
	N2	42
"M"	M0	16
	M1	134
"G"	G1	2
	G2	31
	G3	61
	G4	56
Histological type by Laurén [20]	Intestinal type	117
	Diffuse type	33

Received: June 26, 2008.
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Abbreviations used: GC – gastric cancer; VEGF – vascular endothelial growth factor.

For detection of the E-cadherin, α- and β-catenins, VEGF proteins respective monoclonal antibodies were used (respectively clones NCH-38, α-catenin-1, β-catenin-1 and VG1, Dako Cytomation, Denmark). For visualization of immunohistochemical reaction, Envision+ kit and 3,3-diaminobenzidine (Dako Cytomation, Denmark) were used, with the next staining of slides with hematoxiline.

The tumors were considered positive by studied marker if cytoplasmic reaction was present in > 20% cells.

Statistical analysis of the data was performed with the use of program STATISTICA 6.0. Correlation analysis was done using Pearson association coefficient for patterns that had 2 grades (the presence of distant metastases and metastases in regional lymph nodes, histological type of tumor). For patterns that had more than 2 gradations (the degree of gastric wall injury and metastases in regional lymph nodes, stage of the disease by TNM classification, survival time), Chuprov's coefficient of reciprocal contingency (K) was used. Significancy of mentioned coefficients was evaluated by χ^2 criterium [18]. Patient's survival was analyzed by Kaplan-Mayer's test. Comparison of survival curves was performed with the use of Log-rank-test.

RESULTS AND DISCUSSION

Relation between expression of markers of cell-to-cell adhesion and angiogenesis and GC stage. It is known that the stage of gastric tumors by TNM classification is an important prognostic pattern influencing the course of the disease. As a rule, characteristics of the primary lesion (T) indicates the timeliness of GC diagnosis and allows prescribe an adequate therapy [19]. Upon the study of E-cadherin expression, we have shown an obvious relation between its presence in tumor and GC morbidity (Table 2). The highest expression level of the protein was observed in 79.3% tumor samples of the patients with II stage (Fig. 1, *a*), and is by 38.57% higher ($p < 0.05$), than that in the patients with III stage and by 42.47% higher ($p < 0.05$) than in the patients with stage IV. By our data expression of α-catenin was observed in 51.72% samples of the patients with stage II (Fig. 1, *b*), and is by 20.24% higher ($p < 0.05$) than that in the patients with stage III and by 21.9% higher ($p < 0.05$) than that in the patients with stage IV. The percent of β-catenin-positive tumors has been decreasing progressively dependent on the stage of the disease and was 37.93%, 27.78% and 24.56% in the patients of stages II (Fig. 1, *c*), III and IV respectively. Expression of VEGF in the studied samples was observed as a rule mainly at the late stages (71.93% at stage IV versus 24.14% at stage II (Fig. 1, *d*), and 29.63% at stage III respectively).

Table 2. Correlation between expression of GC molecular markers and the stage of the disease progression (by TNM classification)

Marker	Tumors positive for studied marker, %			K	*p*
	Stage II	Stage III	Stage IV		
E-cadherin	79.31	40.74	36.84	0.29	< 0.01
α-Catenin	51.72	31.48	29.82	0.15	> 0.05
β-Catenin	37.93	27.78	24.5	0.08	> 0.05
VEGF	24.14	29.63	71.93	0.34	< 0.05

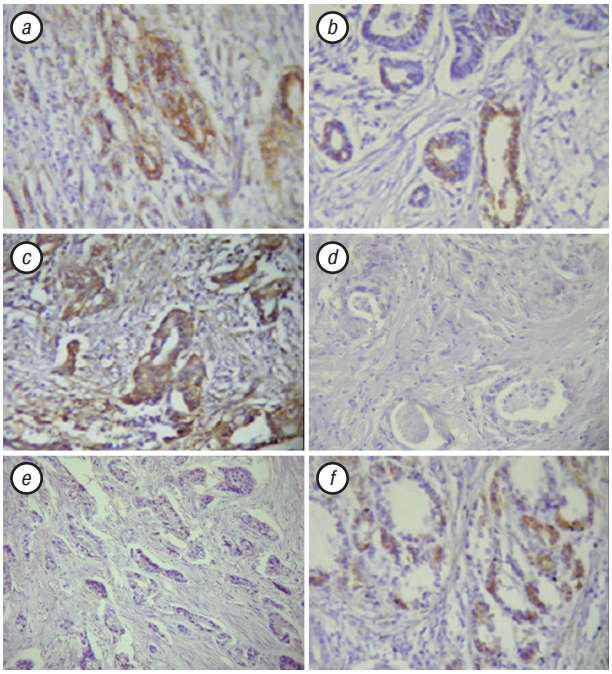

Fig. 1. Immunohistochemaical analysis of VEGF, E-cadherin, α- and β-catenin expression, x 200. *a*, E-cadherin expression in stage II gastric cancer tissue samples; *b*, α-catenin expression in stage II gastric cancer tissue samples; *c*, β-catenin expression in stage II gastric cancer tissue samples; *d*, VEGF expression in stage II gastric cancer tissue samples; *e*, β-catenin expression in samples with metastases in lymph nodes (N_2); *f*, VEGF expression in samples with high degree of gastric wall injury (T_4)

So, we have observed the change of the percent of tumors positive by the studied markers of cell-to-cell adhesion and angiogenesis, dependent on the stage of the disease by TNM classification.

To determine the nature of such changes, we have performed a study of contingency of the relations between the presence of these proteins in gastric tumors and the main characteristics used in TNM classification, namely, the degree of gastric wall injury, the presence of metastasis in lymph nodes, and distant metastases. The results of correlation analysis have demonstrated the existence of an obvious dependence between the presence of expression of markers of cell-to-cell adhesion and angiogenesis in gastric tumor cells and the degree of lymph node metastasis (Fig. 2). The presence of E-cadherin in GC samples correlates with the absence of metastases in regional lymph nodes (N_0). In particular, 74.6% cases without metastases in lymph nodes were E-cadherin-positive, while in N_1 and N_2 cases the level of expression decreased to 26.67% and 16.67%, respectively (Fig. 2, *b*). High content of α-catenin-positive cells was observed in tumor samples from the cases without metastases in regional lymph nodes (61.90 %), and was 5.6 fold higher and 3.24 fold higher than that in N_1 and N_2 cases respectively.

Also we have revealed a tendency for decrease of the percent of β-catenin-positive tumors in the patients with high degree of lymph node metastasis (N_2) (Fig. 1, *e*), compared to that index in the patients with lymph node status N_1 and N_0 (16.67% *vs* 31.11%, and 34.92%, respectively (Fig. 2, *b*). Along with this, it was shown that β-catenin is present in the cases without

distant metastases (43.75%), while in the cases with distant metastases its expression was by 15.39% lower. Expression of VEGF was mainly observed in the GC cases with high degree of gastric wall injury (Fig. 1, *f*) (68.97% positive tumors of T_4 stage *vs* 27.27% and 30.00% positive tumors at T_2 and T_3 stages respectively) (Fig. 2, *a*), and also in the cases with metastasis in regional lymph nodes (53.33% in N_1 cases and 50.00% N_2 cases *vs* 34.92% for the patients without metastases in regional lymph nodes) (Fig. 2, *b*).

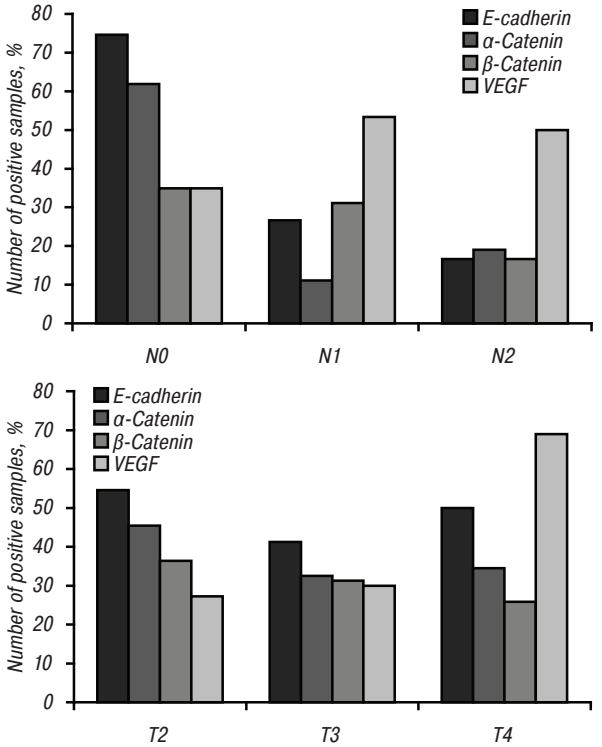

Fig. 2. Distribution of the tumors positive for the studied markers, dependent on the degree of gastric wall injury (*a*) and lymph nodes status (*b*)

Using calculation of Chuprov's coefficient of reciprocal contingency (K), we have reveal correlation between the stage of the disease by TNM classification and the presence of E-cadherin (K = 0.31) and VEGF (K = 0.34) in tumors. Also, the presence of E-cadherin (K = 0.45), α-catenin (K = 0.41) and VEGF (K = 0.14) in tumors correlates with the degree of lymph node metastasis. The presence of VEGF correlates also with the degree of gastric wall injury (K = 0.32).

Relation between expression of cell-to-cell adhesion markers and angiogenesis and histological type of GC. It is known that histological analysis is a key diagnostic method for malignant neoplasia including GC and allows determine a number of important clinico-morphological patterns. Presently, histological classification of GC by Laurén [20] is widely used in clinical practice; it classifies two types of gastric cancer differing in their clinical course and morphogenesis - intestinal and diffuse ones [21].

According to our results (Table 3), intestinal and diffuse GC types differ also by the presence of expression of studied markers. In particular, in intestinal type expression of E-cadherin and β-catenin was detected 2.3- and 3.4-fold more often. α-catenin expression was present in tumors of intestinal type (31.62%), as well as diffuse type (42.42%) without statistical significance. According to our data, hyperexpression of VEGF was observed mainly in tumors of diffuse type (63.64%), in accordance with the data of literature [17, 22] that describe diffuse type of GC as aggressive one and with early metastasis. So, we have demonstrated that there is a dependence between the presence of studied markers and histological type of GC.

Table 3. Correlation between expression of GC molecular markers and histological type of tumors by Laurén classification [20]

Marker	Tumors positive for studied marker, %		r_A	*p*
	Intestinal type	Diffuse type		
E-cadherin	78.79	34.19	0.36	< 0.01
α-Catenin	31.62	42.42	0.08	< 0.01
β-Catenin	63.64	18.80	0.39	< 0.01
VEGF	39.32	63.64	0.19	< 0.01

Prognostic value of adhesion and angiogenesis markers as indexes of survival time of GC patients. The samples of gastric tumors were distributed to three groups dependent on survival time of the patients: 1) survival time < 1 year (n = 77); 2) survival time = 1–3 years (n = 45) 3) survival time > 3 years (n = 28). Distribution of the studied samples positive by studied markers dependent on survival time of the patients are shown in Table 4.

Table 4. Correlation between expression of GC molecular markers and survival of GC patients

Marker	Tumors positive for studied marker, %			K	*p*
	Group 1[1]	Group 2[2]	Group 3[3]		
E-cadherin	16.88	66.67	82.14	0.48	< 0.01
α-Catenin	19.48	31.11	78.57	0.39	< 0.01
β-Catenin	33.77	24.44	21.43	0.10	> 0.05
VEGF	64.94	24.44	21.43	0.35	< 0.01

Notes: [1]survival time < 1 year; [2]survival time = 1–3 years; [3]survival time > 3 years.

We have shown that in the majority of tumors of the patients from group 1 hyperexpression of VEGF and an absence of E-cadherin and α-catenin was observed. In group 2 increase of the percent of E-cadherin-positive tumors (66.67%) and α-catenin-positive ones (31.11%) was detected as well as the decrease of the percent of VEGF-positive tumors (24.44%) compared to group 1. Characteristic pattern of tumors of group 3 was elevated expression of E-cadherin (82.14% cases) and α-catenin (78.57% cases) and low level of VEGF expression (5–10%) only in the 21.43% cases. β-catenin expression was observed in tumors of the patients from all three groups with the tendency for decrease of β-catenin-positive tumors in group 3 compared to group 1 (21.34% and 33.77%, respectively).

Analysis of survival curves by the method of Kaplan — Mayer (Fig. 3). has shown that survival of GC patients was significantly higher in the cases of expression of E-cadherin and a-catenins and in the absence of VEGF expression.

At the base of the results of correlation and comparative analisis of survival curves we have demonstrated that the presence of E-cadherin and α-catenin in gastric tumors as well as the absence of VEGF could be considered as the markers of favorable prognosis by criterium of survival time.

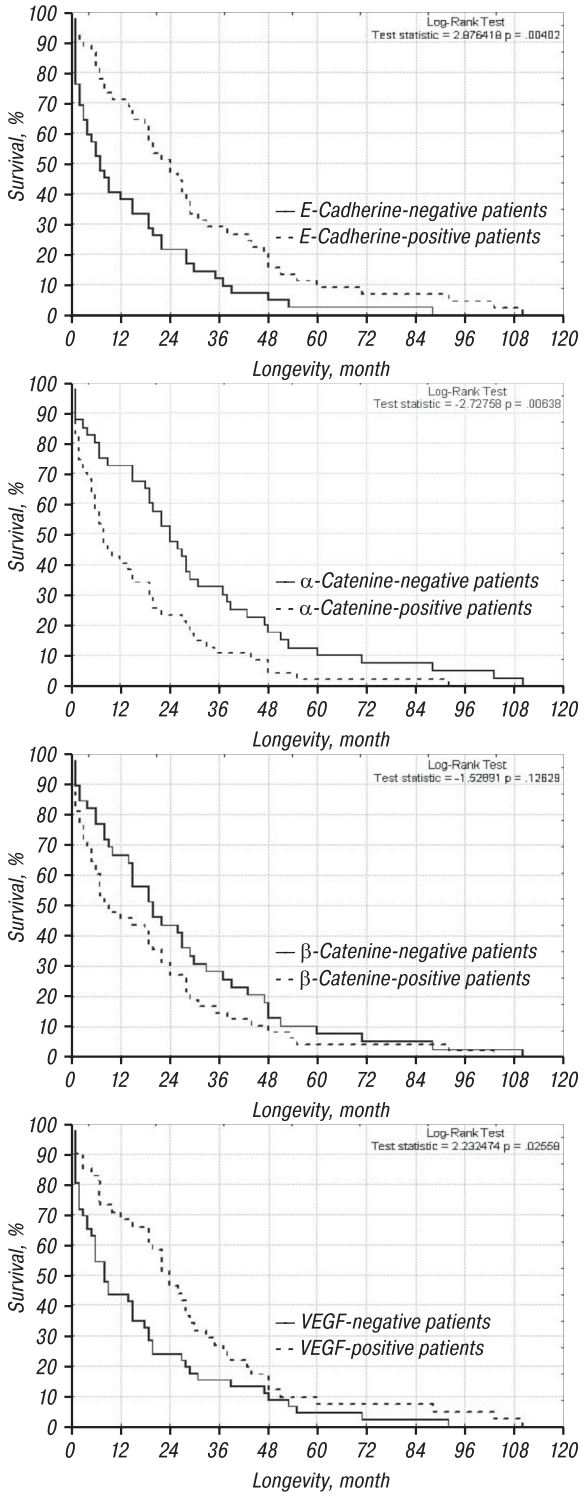

Fig. 3. Kaplan — Mayer distribution of the patients by survival time dependent on the presence of E-cadherin (*a*); α-catenin (*b*); β-catenin (*c*) and VEGF (*d*) in tumor cells

In conclusion, the relation between expression of cell-to-cell adhesion molecules (E-cadherin, α-, β-catenins) and VEGF and traditional clinico-morphological characteristics of tumors and survival time of GC patients has been revealed. We have shown the existence of correlation between the presence of expression of markers of cell-to-cell adhesion and angiogenesis and the stage of the disease. It is demonstrated that the presence of E-cadherin in gastric tumors correlates with the absence of metastases in regional lymph nodes and is observed as a rule at early stages of the disease. the presence of β-catenin expression is characteristic for GC tumors without distant metastases, while the level of VEGF expression correlates with the degree of gastric wall injury. Along with this, the presence of E-cadherin and α-catenin expression in GC is associate with favorable prognosis and is observed in patients with intestinal type of gastric tumors at early stages of the disease. Contrary, VEGF is a marker of unfavorable disease course and its expression is characteristic for diffuse type of GC at late stages of the disease and shorter survival.

So, at the base of combined clinical, histological and immunohistochemical analysis of gastric tumors it has been shown that E-cadherin, α-catenin and VEGF could be used as informative markers of the disease course.

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КОРРЕЛЯЦИЯ МЕЖДУ МЕЖКЛЕТОЧНОЙ АДГЕЗИЕЙ, АНГИОГЕНЕЗОМ И КЛИНИКО-МОРФОЛОГИЧЕСКИМИ ПРОГНОСТИЧЕСКИМИ ФАКТОРАМИ БОЛЬНЫХ РАКОМ ЖЕЛУДКА

Цель: **изучение связи экспрессии молекул межклеточной адгезии (Е-кадгерина, α- и β-катенина) и фактора роста эндотелия сосудов (VEGF) с традиционными клинико-морфологическими характеристиками опухолей для определения их прогностического значения у больных раком желудка.** ***Методы:*** **для определения данных белков в парафиновых срезах тканей пациентов использовали иммуногистохимический метод с использованием моноклональных антител, специфических к Е-кадгерину, α- и β-катенину, а также VEGF.** ***Результаты:*** **присутствие в карциномах Е-кадгерина коррелировало с отсутствием метастазов в регионарных лимфатичнеских узлах и наблюдалось, как правило, у больных на ранних стадиях болезни. Наличие экспрессии β-катенина отмечалось в карциномах желудка тех больных, которые не имели отдаленных метастазов, а уровень экспрессии VEGF четко коррелировал со степенью поражения стенки желудка. Показано, что экспрессия Е-кадгерина и α-катенина ассоциируется с благоприятным прогнозом и является характерным признаком ранних стадий рака желудка кишечного типа. В то же время экспрессия VEGF характерна для поздних стадий рака желудка диффузного типа и указывает на неблагоприятный прогноз.** ***Выводы:*** **в результате комплексного клинического, гистологического и иммуногистохимического анализа рака желудка установлено, что определение Е-кадгерина, α-катенина и VEGF может быть использовано в качестве информативных маркеров течения заболевания.**

Ключевые слова: **рак желудка, прогноз, Е-кадгерин, α-катенин, β-катенин, VEGF.**