Exp Oncol 2011
33, 3, 182–185

Experimental ONCOLOGY

MOLECULAR PROFILE OF GASTRIC CANCER AS A BASIS OF INDIVIDUALIZED TREATMENT AND PROGNOSIS OF DISEASE OUTCOME

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Gastric cancer (GC) was the most frequent type of malignant neoplasm worldwide in the 1980s. Although its incidence has gradually declined in recent years, today GC still is on the fourth place after lung, breast and bowel cancers (14.9 new cases per 100,000 in 2010) [1]. In Ukraine GC is third after lung and skin cancer among men and sixth after breast, skin, endometrial, intestinal and cervical cancer among women.

GC still has a very high mortality rate (second after lung cancer) with 11,4 deaths per 100,000, which continuously declines during last decades. Such high mortality rate is caused by the fact that GC is often diagnosed during late (III–IV) stages, when tumor becomes resistant to chemo- and radiotherapy [2, 3].

It is known that patients with tumors of the same stage and histological type show totally different response to therapy. That is why it is important to find molecular markers which are associated with different aspects of tumor growth and differentiation that would help to predict the disease outcome [1, 4].

Changes in apoptosis and activation of signal cascades are vitally important for tumors. Wild type p53 is able to activate DNA repair proteins when DNA has sustained damage, induce growth arrest by holding the cell cycle at the G1/S regulation point on DNA damage recognition or initiate apoptosis if DNA damage proves to be irreparable. The mutant form of this protein (tp53) is unable to carry out its functions. Such changes in p53 structure are observed in more than 50% of tumors of different origin. Another important regulator of apoptosis is BCL-2 protein which has an ability to inactivate different proapoptotic proteins [1, 2].

EGFR and HER-2/neu are tyrosine kinase receptors from the ErbB family which are often overexpressed on the tumor cell membrane. These receptors are activated by different ligands by homo- or heterodimerisation. The signal from intracellular tyrosine kinase domain is then transduced to the NF-κB, AKT and ERK signal pathways, phosphatydilinositol-3-kinase or phospholipase C, what causes changes in apoptosis, cell division and differentiation [5].

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Abbreviations: EGFR – epidermal growth factor receptor; GC – gastric cancer; mAb – monoclonal antibody; VEGF – vascular endothelial growth factor.

Adhesion cell contacts formed by cadherin-catenin complex (E-cadherin, α- β- and γ-catenins) play important role in GC growth. Intracellular domains of E-cadherin are bound to β-catenin, γ-catenin and p120ctn protein forming a cytoplasm cell adhesion complex, which is necessary for formation of adhesive cellular junctions. β-catenin and γ-catenin are also bound to α-catenin, which connects cadherin-catenin complex with actin cytoskeleton. Loss of any of these proteins increases the risk of appearance of local or distant metastases [2, 6].

Vascular endothelial growth factor (VEGF) induces formation of protrusions in vascular walls, through which proteins could move out of the blood vessels. As a result, extravascular fibrin gel which mediates growth of endothelial cells, is formed. This causes blood vessels to grow into the tumor body, thus promoting its growth. On the other hand, tumor which has high microvessel density could be more sensitive to chemotherapy [1–3].

So, the aim of the study was to perform complex study of relations between clinico-morphological prognostic factors and expression of regulators of apoptosis (p53, bcl-2), tyrosinkinase receptors (EGFR, HER-2/neu), adhesion molecules (E-cadherin, α- and β-catenins) and VEGF.

We have analyzed tumor samples from 150 GC patients using classic immunohistochemical method. Sex, age of Gc patients and GC stage distribution corresponded to the statistical data for the Ukrainian population. Differences between groups were studied using Student's *t*-test, correlations were studied with computing Pierson's (r) and Chuprov's (K) correlation coefficients.

On the first stage of the study we have analyzed an expression of regulators of apoptosis, tyrosine kinase receptors, markers of cell adhesion and neoangiogenesis in relation to generally accepted clinico-morphological tumor features (stage, depth of invasion, local and distant metastases).

We revealed that 75.4 ± 4.0% of stage IV GC patients showed positive nuclear reaction with mAb specific to tp53, while only 24.1 ± 5.6 and 31.5 ± 4.4% of patients with stage II and stage III GC, respectively, had tp53-positive tumors (Table 1). In the meantime Bcl-2 expression was predominantly found in tumors of stage II patients (72.4 ± 5.8 *vs.* 14.8 ± 3.4 % and

15.8 ± 3.4% for stage III and IV, respectively). Also we have found moderate correlation between the stage of the disease according to TNM and tp53 (K=0.34, $p<0.01$) and Bcl-2 (K=0.32, $p<0.01$) expression. Another interesting finding was moderate correlation (r=0.32, $p<0.01$) between the presence of Bcl-2 protein and absence of tp53 in the tumor.

Table 1. Correlations between expression of molecular markers of apoptosis, angiogenesis, cell adhesion and tyrosine kinase receptors in GC tumors and TNM stage of the disease

Marker	Stage % of positive tumors			K	*P*
	II	III	IV		
p53	24.1 ± 5.6	31.5 ± 4.4	75.4 ± 4.0	0.34	<0.01
Bcl-2	72.4 ± 5.8	14.8 ± 3.4	15.8 ± 3.4	0.32	<0.01
E-cadherin	79.3 ± 5.3	40.7 ± 4.7	36.8 ± 4.5	0.31	<0.01
α-catenin	51.7 ± 6.5	31.4 ± 4.4	29.8 ± 4.2	0.15	>0.05
β-catenin	37.9 ± 6.3	27.7 ± 4.3	24.5 ± 4.0	0.08	>0.05
EGFR	34.4 ± 6.2	29.6 ± 4.3	33.3 ± 4.4	0.06	>0.05
Her-2/neu	17.2 ± 4.9	27.7 ± 4.3	49.1 ± 4.6	0.30	<0.01
VEGF	24.1 ± 5.6	29.6 ± 4.3	71.9 ± 4.2	0.34	<0.01

We also found that E-cadherin, α- and β-catenin expression in GC tumors was often associated with earlier stages of the disease. Particularly, 79.3 ± 5.3 % of stage II tumors were E-cadherin-positive (K=0.31), 51.7 ± 6.5% — α-catenin-positive and 37.9 ± 6.3% — β-catenin-positive. Statistical analysis showed significant correlations only between the GC stage and E-cadherin expression (K=0.31, $p<0.01$).

Other markers that showed significant differences between different GC stages were Her-2/neu and VEGF. Presence of both of these proteins in tumors were associated with the stage IV of the disease (49.1 ± 4.6 and 71.9 ± 4.2% of positive tumors, respectively). We also showed moderate correlation between VEGF expression and tumor stage (K=0.34, $p<0.05$).

Next, we have studied the expression of mentioned proteins in relation to parameters on which TNM classification is based: T (depth of gastric wall invasion), N (local metastases), M (distant metastases). We didn't observe any significant differences in expression of studied proteins in relation to M status because of very small group of M-positive patients (n = 16).

We found that both studied regulators of apoptosis (tp53 and Bcl-2) and VEGF were expressed mostly in lymph-node positive GC tumors (55.2 ± 2.6%, 48.4 ± 2.4% and 51.3 ± 2.4%, respectively). Also, we observed increase of Her-2/neu expression from 28.5 ± 1.7% in N_0 tumors to 40.5 ± 2.1% in tumors with N_2. On the other hand, E-cadherin, α- and β-catenin–positive tumors were present in patients without regional lymph node metastases (74.6 ± 3.1% for E-cadherin and 61.9 ± 2.5% for α-catenin). We found moderate correlations between E-cadherin (K=0.45, $p<0.05$), α-catenin (K=0.41, $p<0.05$), and VEGF (K=0.41, $p<0.01$) expression and lymph node status.

The only protein, which expression significantly differed between groups of patients with different T-stage tumors was VEGF. Its expression correlated (K=0.32, $p<0.01$) with T_4 stage of gastric wall invasion (68.9 ± 3.2% of positive tumors).

Another significant criterion in diagnostics of neoplasms of any localization is their morphological investigation. Today Lauren's GC classification [7] is often used in clinical practice, according to which two types of the disease are diagnosed: intestinal (slow growth, high degree of differentiation) and diffuse (faster growth, lower degree of differentiation).

We have found (Table 2) that E-cadherin and β-catenin were associated with GC of intestinal type (78.7 ± 5.0 and 63.6 ± 5.9%, respectively) which is thought to be more favorable in prognosis because of higher sensitivity to chemotherapy. Also positive expression of both studied tyrosine kinase receptors was related to this histological type of GC (69.7 ± 5.6% for EGFR and 72.7 ± 5.4% for Her-2/neu). Statistical analysis showed moderate correlations between E-cadherin (r=0.36, $p<0.01$), β-catenin (r=0.39, $p<0.01$), EGFR (r=0.42, $p<0.01$) and Her-2/neu (0.50, $p<0.01$) expression and histological type of tumor. On the other hand, tp53 and VEGF positive expression were markers of diffuse GC type (r=0.48 and r=0.19, respectively).

Table 2. Correlations between expression of molecular markers of apoptosis, angiogenesis, cell adhesion and tyrosinkinase receptors in GC tumors and histological type of the disease

Marker	Histological type of tumor % of positive tumors		r	*p*
	Intestinal type	Diffuse type		
p53	17.9 ± 2.5	72.7 ± 5.4	0.48	<0.01
Bcl-2	54.5 ± 6.1	46.1 ± 3.2	0.05	<0.01
E-cadherin	78.7 ± 5.0	34.1 ± 3.1	0.36	<0.01
α-catenin	42.4 ± 3.0	31.6 ± 6.0	0.08	<0.01
β-catenin	63.6 ± 5.9	18.8 ± 2.5	0.39	<0.01
EGFR	69.7 ± 5.6	20.5 ± 2.6	0.42	<0.01
Her-2/neu	72.7 ± 5.4	22.2 ± 2.7	0.50	<0.01
VEGF	39.3 ± 5.9	63.6 ± 3.1	0.19	<0.01

One of the main prognostic criteria of treatment efficiency is the duration of the survival period. We divided tumor samples into three groups according to the length of patient survival period (Group 1 — less than 12 months, Group 2 — 12–36 months and Group 3 — more than 36 months).

We showed that tumors from the patients from the Group 1 expressed VEGF (64.9 ± 3.9 %), Bcl-2 (41.5 ± 3.9%) and tp53 (67.5 ± 3.7%) and were mostly E-cadherin and α-catenin negative (16.8 ± 3.0% and 19.4 ± 3.1% of positive tumors, respectively). Patients from the Group 2 were characterized by increased number of E-cadherin-positive (up to 66.6 ± 4.9%) and α-catenin-positive (31.1 ± 4.8%) tumors, while number of VEGF-positive tumors decreased (24.4 ± 4.5%). The Group 3 of patients was characterized by highest percent of E-cadherin- and α-catenin-positive tumors (82.1 ± 5.1 % and 78.5 ± 5.4 %). Also, low and moderate β-catenin, Her-2/neu and EGFR expression were observed in all three groups of patients.

We found correlations between tp53 (K=0.34, $p<0.01$), Bcl-2 (K=0.30, $p<0.01$), E-cadherin (K=0.48, $p<0.01$), α-catenin (K=0.39, $p<0.01$) and VEGF (K=0.35, $p<0.01$) and length of the survival period.

We also built Caplan — Meier survival curves (Figure) for groups of patients with tumors positive or negative by each studied marker. Analysis of these curves confirmed the results mentioned above and showed that patients with tp53-negative, Bcl-2-negative, E-cadherin-positive, α-catenin-positive and

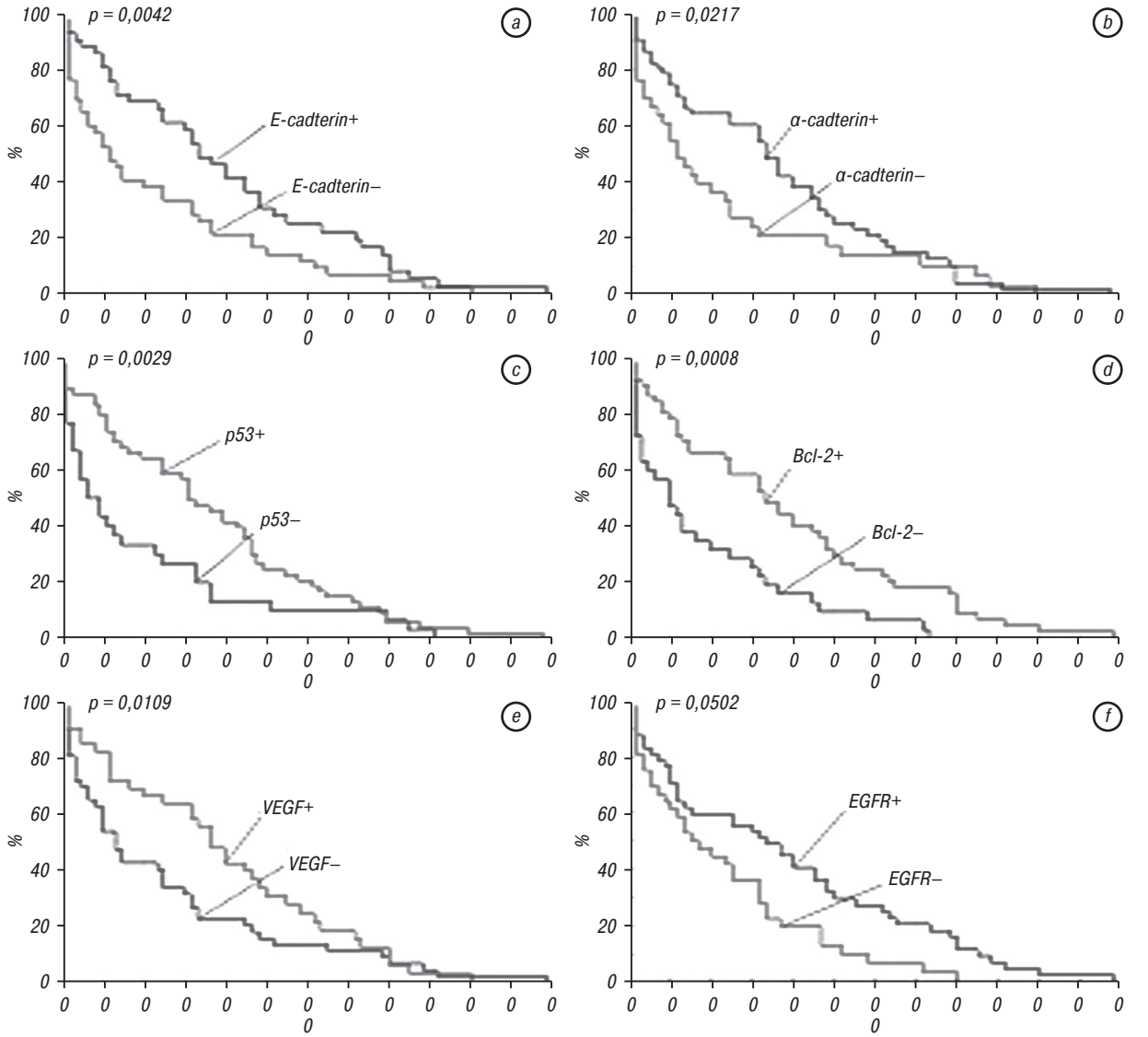

Figure. Caplan — Meier survival curves for GC patients with tumors positive and negative by E-caddherin (A), α-catenin (B), tp53 (C), Bcl-2 (D), VEGF (E) and EGFR (F) expression. Curves were compared using Wilcoxon U-test

VEF-negative tumors had significantly longer overall survival period while we did not observe significant differences between patients with β-catenin, EGFR and Her-2/neu-positive and negative tumors.

Multifactor Cox analysis (Table 3) showed that p53, Bcl-2, E-cadherin, α-catenin and VEGF positive expressions could be used as independent prognostic factors. Particularly, E-cadherin and α-catenin positive expression, as well as loss of p53, Bcl-2 and VEGF expression are markers of favorable disease prognosis and correlates with longer survival period.

Table 3. Multifactor Cox analysis of studied markers

Marker	β	*P*
p53	-0.33	<0.05
Bcl-2	-0.28	<0.05
E-cadherin	0.46	<0.05
α-catenin	0.51	<0.05
β-catenin	-0.10	>0.05
Her-2/neu	-0.14	>0.05
EGFR	-0.08	>0.05
VEGF	-0.36	<0.05

In conclusion, we showed strong correlation between expression of p53, Bcl-2, E-cadherin, Her2/neu and VEGF in tumor cells and the stage of disease according to TNM classification. We observed the highest percent of p53, Her-2/neu and VEGF positive tumors in group of stage IV GC patients, while high levels of Bcl-2 and E-cadherin were observed in tumors of stage II GC patients. GC patients with T4 degree of gastric wall invasion show tendency to increased percent of Bcl-2- and VEGF-positive tumors. Also we showed that E-cadherin expression correlated with absence of lymph node metastases and is observed primarily in GC patients with II stage. β-catenin expression is more often observed in GC tumors without distant metastases, while VEGF expression correlated with T-stage and presence of metastases in regional lymph nodes. Expression of some molecular markers is associated with histological type and degree of tumor differentiation. We observed increase of E-cadherin, α-catenin, EGFR and Her2/neu-positive tumors in the group of patients with intestinal type of GC. Tumors of diffuse type often expressed p53 та VEGF. We proved that the presence of p53, Bcl-2, E-cadherin, α-catenin and VEGF could be used as independent prognostic markers of survival for GC patients: high E-cadherin,

α-catenin and low p53, Bcl-2 and VEGF expression in tumor cells point on favorable prognosis of disease outcome with significantly longer survival period.

REFERENCES

1. **Cappellani A, Zanghi A, Di Vita M, *et al.*** Clinical and biological markers in gastric cancer: update and perspectives. Front Biosci (Schol Ed) 2010; **1**: 403–12.

2. **Catalano V, Labianca R, Beretta GD, *et al.*** Gastric cancer. Crit Rev Oncol Hematol 2005; **3**: 209–41.

3. **Dicken BJ, Bigam DL, Cass C, *et al.*** Gastric adenocarcinoma. Review and considerations for future directions. Ann Surg 2005; **1**: 27–39.

4. **Yeoh KG.** How do we improve outcomes for gastric cancer? J Gastroenterol Hepatol 2007; **7**: 970–2.

5. **Dumanskij YV, Vlasenko DL, Balashova OI, *et al.*** Possibility of using HER-2/NEU & KI-67 as prognostic factors of distal stomach tumor answer for neoadjuvant therapy. Morphology 2008; **2**: 51–6. (In Russian).

6. **Concolino P, Papa V, Mozzetti S, *et al.*** The unsolved enigma of CDH1 down-regulation in hereditary diffuse gastric cancer. J Surg Res 2004; **1**: 50–5.

7. **Laurén P.** The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 1965; **64**: 31–49.