Expression of EpCAM protein in gastric cancer cells may contribute to its histogenesis

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ABSTRACT

Introduction: EpCAM protein belongs to adhesion molecules of epithelial cells. It mediates in the homophile adhesion cell-cell reactions. EpCAM protein expression can be observed in the majority of healthy normal cells. However, mutations in *EpCAM* gene may lead to an increased risk of cancer development. The aim of the study was to assess EpCAM protein expression in the correlation with chosen clinical and histological parameters in gastric cancer.

Materials and Methods: EpCAM protein expression was evaluated immunohistochemically in 88 patients diagnosed with gastric cancer.

Results: An increase in EpCAM protein expression was demonstrated in cancer cells compared to normal gastric mucosa (59.3% cancers with the positive expression of EpCAM protein). The increased EpCAM protein expression was observed in patients with a histological type of adenocarcinoma without a mucinous component than in those with adenocarcinoma with a mucinous component (p=0.028). The higher expression of this protein was observed also in the intestinal type according to the Lauren classification (p=0.037). The expression of the protein was lower in the diffuse type of cancer. Additionally, an increase in EpCAM protein expression was revealed in cancers infiltrating to the blood vessels (p=0.013).

Conclusions: A correlation between EpCAM expression and adenocarcinoma without a mucinous component as well as the intestinal type according to the Lauren classification may prove a role of this protein in the histogenesis of gastric cancer. Moreover, its positive expression is related to cancerous cells infiltrating to the blood vessels, which may suggest a role of EpCAM protein in the early stages of gastric cancer metastases.

Keywords: Adhesion, EpCAM, gastric cancer

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INTRODUCTION

Gastric cancer is a malignant cancer deriving from glandular epithelium. The annual morbidity equals a million people of whom 760 000 cases are lethal [1].

EpCAM (epithelial cell adhesion molecule) protein is an adhesion molecule of epithelial cells which mediates in the homophile interactions of cell – cell [2]. These combinations are relatively weak in comparison with other adhesion molecules, such as cadherins. EpCAM was discovered for the first time as a superficial antigen in colon cancer [3].

This protein can be seen in the membrane layer of the cells. The expression level of EpCAM is various in different cells: the highest is observed in the small intestine and colon, the lower in the stomach [4]. Though EpCAM belongs to a big family of adhesion proteins, it is structurally similar to none of four families of adhesion molecules: cadherins, integrins, selectins and a superfamily of immunoglobulins. It is built out of three domains: intracellular domain, defined also as EpICD, endothelial domain as well as extracellular domain, called EPEX [5, 6]. EpICD domain plays the most significant role in the cellular adhesion [7]. EpCAM influences negatively bondings where cadherins mediate. When EpCAM expression increases the amount of α-catenin decreases but the level of β-catenin does not change [7]. EpCAM can be broken apart and thus, gaining an oncogenic potential. An extracellular domain is released to the area surrounding the cell, while an intracellular domain is released to the cytoplasm. EpICD combines with FHL2, β-cathenin and Lef proteins inside the nucleus. The complex is forms, which combines with DNA, intensifying the protein expression of some genes: c-myc, E-FABP, A and E cycline [9]. EPEX may intensify the breakage of the next EpCAM molecules, followed by the phenomenon of a positive feedback leading to breaking more and more numerous molecules of the protein [6].Due to the role of EpCAM in maintaining the cells integrity, the aim of this study was to establish a role of this protein in gastric cancer. Moreover, the study focused on the possibility of using EpCAM as a potential prognostic marker in gastric cancer.

MATERIALS AND METHODS

The study was carried out among 88 patients diagnosed with gastric cancer. The material was obtained during surgical resection of the tumour. The healthy gastric mucosa taken from the material resected during operation constituted the control group. The postoperational material was preserved in buffered formalin and embedded in the paraffin. Routine histopathological analysis included determination of tumor histological type, malignancy grade (G), anatomoclinical stage (pT), and lymph node metastases. Gastric cancers were also divided according to Lauren's classification. Also, the presence of Helicobacter pylori infection was assessed in Giemsa stained preparations.

In accordance with the GCPs (Guidelines for Good Clinical Practice), this research was approved by the Bioethical Commission of the Medical University of Bialystok (Resolution No.: R-I-002/210/2015).

Immunohistochemical analysis. Paraffin blocks with tissues were cut on the microtome into slides of 4μm thick on the silanized slides. The pieces were deparaffinised in the xylens and hydrated in the alcohols.

Next they were placed in citric buffer (pH=6.0) and incubated in the aqueous bath for 20 minutes, at the temperature of 98.5^{o}C to reveal the antigen and then for 20 minutes in room temperature.

Next, incubation was performed with a 3% hydrogen peroxide to block endogenous peroxidase and a 1% bovine serum to block non- specific bonds. In the next stage, the slides were incubated with rabbit monoclonal antibody anti-EpCAM (Sigma Aldrich, HPA026761, dilution 1:200) for 30 minutes at room temperature.

After the reaction in the polymeric technique (Novocastra), the complex antigen-antibody was exposed due to the usage of chromogen 3.3'-diaminobenzidine (DAB, Novocastra, UK). The cellular nuclei were stained with haematoxylin.

The immunohistochemical reaction was assessed via calculating neoplastic cells with the positive expression of the protein in 10 representative fields. The mean value of the expression was shown in percentage. The evaluation was performed with a light microscope using magnification of 400x.

Statistical analysis was performed based on the Spearman's correlation coefficient test.

The value of $p<0.05$ was considered as statistically significant. Statistics was performed by means of STATISTICA 12 (Poland) programme.

RESULTS

In cancerous cells, a higher expression of EpCAM protein was observed (59.3% of cancers with a positive expression of EpCAM protein) in comparison with normal gastric mucosa (Figure 1).

The increased EpCAM protein expression was demonstrated in patients with a histological

type of adenocarcinoma without a mucinous component than in those with adenocarcinoma with a mucinous component (p=0.028) (Table 1).

Table 1. Correlation between clinico-histopathological parameters and EpCAM protein expression.

Variables		N (number of cases)	Mean expression EpCAM (%)	Coefficient R *p* value
Age	<60	27	29.8	-0.036 0.212
	≥60	61	24.2	
Gender	Female	27	16.7	0.179 0.099
	Male	61	29.8	
średnica guza	<5 cm	20	17.9	0.126 0.246
	≥5 cm	68	28.1	
Location	Upper 1/3	16	22.8	-0.012 0.911
	Middle 1/3	30	29.5	
	Lower 1/3	17	24.4	
	Whole stomach	25	24.6	
Histological type	Adenocarcinoma	55	32.2	**-0.237** **0.028**
	Adenocarcinoma mucinosum	33	15.8	
Lauren's classification	Intestinal type	49	33.4	**-0.229** **0.037**
	Diffuse type	23	23.4	
	Mixed type	13	8.1	
Histological differentiation	Moderately differentiated	27	32.9	-0.188 0.082
	Poorly differentiated	33	27.8	
	Non differentiated	28	17.0	
Depth of invasion	T1	7	22.1	0.052 0.634
	T2	7	7.86	
	T3	65	29.4	
	T4	9	17.8	
Lymph node metastasis	Absent	18	22.2	0.055 0.609
	Present	70	26.8	
Distant metastasis	Absent	63	26.9	-0.047 0.667
	Present	25	23.4	
Lymphatic vessels infiltration	Absent	24	32.8	-0.019 0.372
	Present	47	25.0	
Blood vessels infiltration	Absent	52	23.8	**0.317** **0.013**
	Present	10	53.0	
Helicobacter pylori infection	Absent	65	26.5	-0.014 0.893
	Present	22	25.0	

Significant relationship is marked in bold. Missing data were removed in pairs.

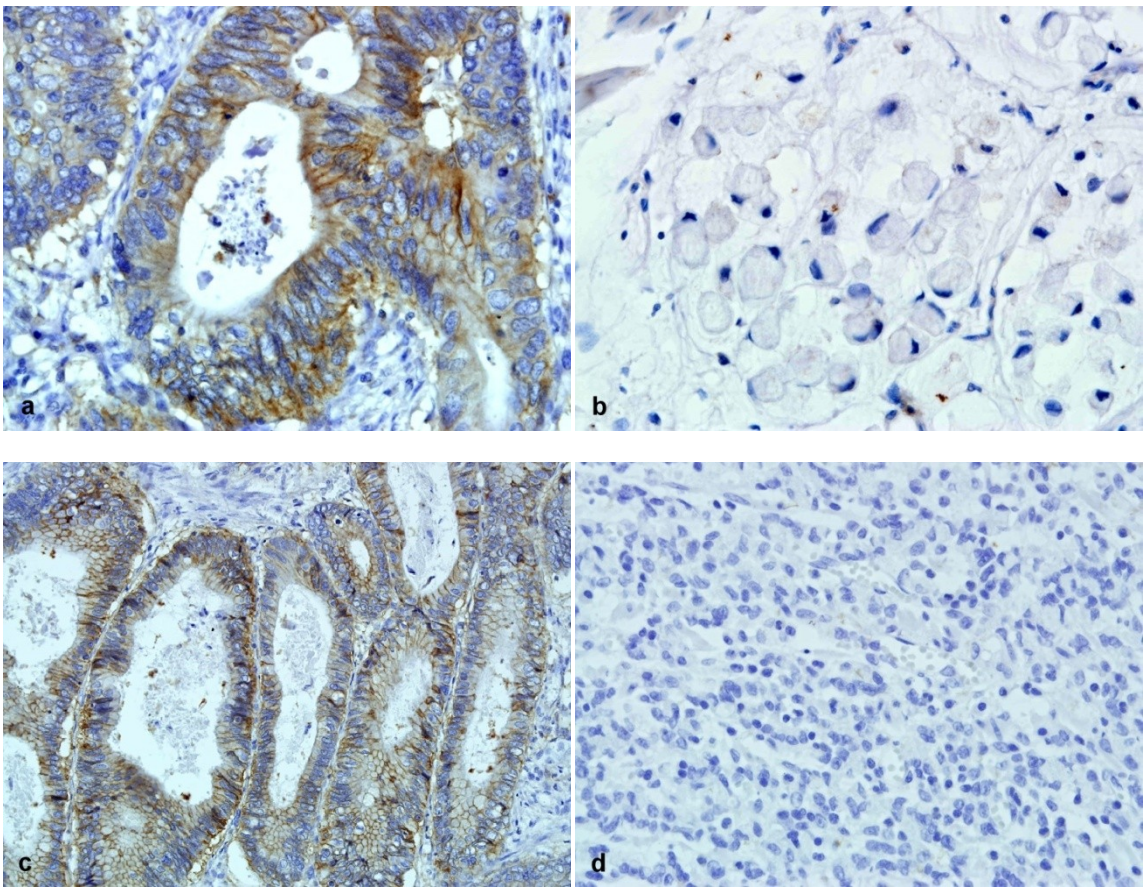

Figure 1. Positive expression of EpCAM in adenocarcinoma without mucosal component (a) and negative expression in mucinous adenocarcinoma (b). Positive expression of EpCAM protein in the intestinal type (c) and negative in a diffuse type (d) of gastric cancer according to Lauren classification. Magnification 400x.

In patients with adenocarcinoma without a mucinous component, the mean EpCAM expression equalled 32.2%, while patients with adenocarcinoma with a mucinous component had the lower EpCAM expression (15.8%) The higher expression of this protein was found in the intestinal type of cancer (33.4%) according to the Lauren classification (p=0.037). However, EpCAM expression was lower in the diffuse type (23.4%) and the lowest in the mixed type (8.1%). Additionally, an increase in EpCAM expression was revealed in cancers infiltrating to the blood vessels (p=0.013). In infiltrating cancers, the mean expression equalled 53.0%, whereas in non-infiltrating cancers, it was significantly lower and totalled 23.8%.

No statistically significant correlations were demonstrated between the expression of EpCAM protein and the patients' age, infiltration depth, infiltration to the lymph nodes, distant metastases, the advancement of cancer, patients' survival rate and *Helicobacter pylori* infection.

DISCUSSION

The over-expression of EpCAM protein was proved in the majority of neoplasms, among the others, in eosophageal cancer, pancreatic cancer and gastric cancer [10-12]. In our study, an increase in the expression of EpCAM protein was proved in cancerous cells compared to normal gastric mucosa. EpCAM protein plays no unequivocally defined role in gastric cancer. Du et al. [13] proved that an increase in EpCAM expression occurred during epithelial-mesynchemal transformation (EMT), while its expression decreased in cancer metastases. However, the studies of Songun et al. [14] demonstrate a positive expression of EpCAM protein in 93% of patients with gastric cancer and a decreased expression during EMT process. Similarly, Santisteban et al. [15] in their studies of EMT cells in breast cancer observed the decreased EpCAM expression. It is expected that EpCAM

expression decreases during EMT to increase again after reaching the target site of metastasis [16]. However, the inconsistent results of the studies unable to define unequivocally the role of EpCAM in the neoplasm process.

In our studies, the higher expression of EpCAM was revealed in gastric cancer without a mucinous component. In their studies, Wang et al. [12] compared also the expression of this protein with a histological type; however , their results were not statistically significant. Additionally, it was proved that the higher expresssion of EpCAM protein occurred in the intestinal type according to the Lauren type classification and when compared to the disseminated and mixed type. Kroepil et al. [17] as well as Joo et al. [18] published the studies where they proved that an increase in EpCAM protein was also found in the intestinal type of gastric cancer. On the other hand, Wang et al. [12] proved a higher expression of EpCAM protein in the disseminated type. They also proved the correlation indicating the shorter survival rate in patients with a higher expression of EpCAM both in the intestinal type and the disseminated type of gastric mucosa. Moreover, Joo et al. [18] proved that an increase in EpCAM protein expression occurred in the well-differentiated cancers. Additionally, they observed that EpCAM protein may take part in the differentiation of cancer into a given histological type. In their studies, they established that EpCAM protein could not be assigned the prognostic role in gastric cancer. In our study, the higher expression was also found in less differentiated cancers. However, the results of our studies were not statistically significant. On the other hand, Du et al .[19] proved the increased expression of EpCAM in poorly differentiated cancers. All these studies may prove that the expression of EpCAM protein might depend on the histological type of gastric cancer.

In our study, no significant correlations were revealed between EpCAM protein expression and parameters showing the advanced stage of neoplasm, such as the depth of infiltrating primary tumour (pT). In our study, no significant correlations were proved between the protein expression and parameters showing the cancer advancement, such as: metastases to the lymph nodes and distant metastases. However, many authors found the increased expression of EpCAM protein in cancers of a higher stage (T3 and T4) [12, 20]. Moreover, it was proved that the higher expression of EpCAM protein occurred in gastric cancer with metastases to the lymph nodes [12, 21]. It was found that the patients with metastases to lymph nodes and a high expression of the protein were proved to have a lower survival rate of 5 years than the patients with a low expression of this protein [12]. Additionally Wang et al. [12] revealed a higher expression of this protein in cancers with distant metastases. Based on the studies performed, they assumed that EpCAM protein played a role in the development of gastric cancer and its metastasis, and can be an independent prognostic factor and an indicator of the survival rate.

In our study, an increase in EpCAM expression was revealed in tumours with cancerous cells infiltrating to the blood vessels. This may confirm its positive role in promoting metastases and indirectly in the development of cancer. However, in the literature there are no findings about the role of EpCAM in gastric cancer cells infiltrating to the blood vessels. Nevertheless, Wang et al.[22] published the study showing a positive cytoplasmic EpCAM expression in colon cancer infiltrating to the blood vessels.

CONCLUSIONS

Summing up, our study proved a significant correlation between the expression of EpCAM protein and gastric adenocarcinoma without a mucinous component as well as an intestinal type in the Lauren classification, which may confirm the role of EpCAM in the histogenesis of gastric cancer. Concurrently, its positive expression is related to cancerous cells infiltrating the blood vessels, which may suggest the role of this protein in the development and metastasis of gastric cancer. Numerous discrepancies are found in the study results published by many authors, which can be explained by the kind of the group examined (race, life style, environmental factors and genetic factors). Thus, the examinations require further research including also genetics.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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