



Overexpression of epithelial cell adhesion molecule (EpCAM) in gastric cancer and its correlation with overall survival of the patients

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Original Article

Abstract

BACKGROUND: Epithelial cell adhesion molecule (EpCAM) is an adhesion molecule which is expressed on the epithelial cells and primarily identified as a tumor marker for carcinoma. In this study, the expression of EpCAM in precancerous and cancerous gastric lesions was investigated and then, the association of EpCAM expression with the overall survival of patient suffering from gastric carcinoma was evaluated.

METHODS: 12 gastric carcinoma, 3 dysplasia, and 8 intestinal metaplasia (IM) subjects were taken from the department of pathology of Tohid Hospital, Sanandaj, Iran. The diagnosis was made by the expert pathologist. Then, the subjects were stained for EpCAM by immunohistochemistry (IHC) and analyzed by the pathologist.

RESULTS: The data showed that EpCAM was expressed in all of the precancerous and cancerous samples. However, 76.4% of carcinoma cells were positive for EpCAM while it was 62.5% and 51.3% for dysplasia and IM, respectively. Importantly, it was observed that the expression of EpCAM on gastric cancer was negatively correlated with the overall survival of the patients.

CONCLUSION: In conclusion, it was demonstrated in this study that EpCAM is expressed in gastric carcinoma and its expression is negatively correlated with the overall survival of the patients with gastric cancer.

KEYWORDS: Epithelial Cell Adhesion Molecule, Gastric Cancer, Tumors

Date of submission: 10 Mar. 2018, **Date of acceptance:** 18 Sep. 2018

Citation: Mohammadi M, Jalili A, Nikkhoo B, Roshani D, Tari K, Sheikhesmaili F. **Overexpression of epithelial cell adhesion molecule (EpCAM) in gastric cancer and its correlation with overall survival of the patients.** Chron Dis J 2019; 7(1): 49-52.

Introduction

Gastric cancer is the second most common cause of cancer mortality worldwide¹ and its incidence increases after the age of 40 years old. The ethnic origin can be a possible risk factor for gastric cancer, as its overall survival and incidence rates vary in different geographic locations, with high incidence in East, South, and Central Asia, Central and Eastern Europe, and South America, whereas the United States has the lowest incidence

rate.^{2,3} Gastroesophageal reflux disease (GERD) and obesity are the main risk factors for the development of proximal tumors, while the major risk factor for distal cancers is dietary factors and Helicobacter pylori (H. pylori) infection.⁴ In spite of remarkable progress in the therapeutic methods and surgical techniques, the outcome of patients with gastric cancer is not satisfactory.⁵ Therefore, multimodal therapy may be a good option for these patients, albeit the lack of effective markers.

The epithelial cell adhesion molecule (EpCAM, CD326) is a 39-42 KDa, 314-amino acid, type I transmembrane glycoprotein

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encoded by the 9-exon gene TACSTD1. This glycoprotein comprises a large extracellular domain with an epidermal growth factor (EGF)-like domain and a putative thyroglobulin (TY) domain, a single transmembrane region, and a short (26 amino acids) cytoplasmic tail. EpCAM belongs to the family of adhesion molecules, but cell adhesion is not its single role and has many other functions including cellular signaling, proliferation, cell migration, and differentiation.^{1, 5-7} EpCAM is overexpressed in various epithelial cancers and its expression has been shown in numerous epithelial tissues.⁸ EpCAM overexpression has been revealed in gastric cancers, while it is not overexpressed in healthy gastric tissues.⁹ In the majority of cancers, EpCAM expression has not a good prognosis, however gastric cancer is accompanied by some contradictions.¹⁰ In this introductory study, EpCAM expression on gastric precancerous and cancerous lesions were examined.

Materials and Methods

Paraffin-embedded tissue samples of 23 cases [12 gastric cancers, 3 dysplasia, and 8 intestinal metaplasia (IM)] were taken out from the archives of the pathology department of Tohid Hospital, Sanandaj, Iran. All samples were confirmed and staged in the pathology department, according to Gleason scores. Clinical information was obtained from the medical records.¹⁰

Immunohistochemistry (IHC): The blocks were cut in 5 μ m and after heating at 60 °C for 1 hour, they were deparaffinized in xylene. Then, the blocks were rehydrated in increasing grads of ethanol. For antigen retrieval, the sample was incubated for 20 minutes at 95 °C in citrate buffer and incubated with 0.3% hydrogen peroxide for 10 minutes at room temperature to block endogenous peroxidase activity. After protein blocking with bovine serum albumin (BSA) 1% for 5 minutes at

room temperature, the samples were incubated with 1:100 diluted EpCAM primary antibody (eBioscience™), at 4 °C for 24 hours. IHC staining was performed with a kit (Dako, Denmark), according to the manufacturer protocol, and hematoxylin was used for counterstaining. After mounting, slides were examined by a pathologist. Only membranous staining was considered for IHC scoring. For each tumor sample, staining intensity (0, 1+, 2+, and 3+) and percentage of positive tumor cells were estimated. Results were grouped as follows: negative (total absence of staining), weak (1+ staining in < 60% of cells or 2+ staining in < 30% of cells), moderate (1+ staining in \geq 60% of cells, 2+ staining in 30% to 70%, or 3+ staining in < 30%), and strong (2+ staining in > 70% or 3+ staining in \geq 30%).¹¹

One way analysis of variance (ANOVA) with Tukey's post-test were used to analyze the statistical differences of EpCAM expression of IM, gastrointestinal dysplasia (GID), and carcinoma. The Kaplan-Meier method was exploited for survival curves, and P values were calculated using the log-rank (Mantel-Cox) test. P values < 0.05 were considered to be statistically significant. All statistical analyses were performed using the SPSS software (version 18.0, SPSS Inc., Chicago, IL, USA).

Results

EpCAM expression: EpCAM expression was evaluated in 23 samples containing 12 carcinomas, 3 GID, and 8 IM. In normal gastric tissues of all samples, no EpCAM expression was seen, however in all of the GID, IM, and carcinomas samples, EpCAM overexpression was found. In the carcinoma samples, 76.4% of cells were positive for EpCAM, whereas in the GID and IM samples, this rate was 62.5% and 51.3%, respectively (Figure 1). The average staining intensity was 3+ in more than 80% of carcinoma cells and 2+ in GID and IM cells.

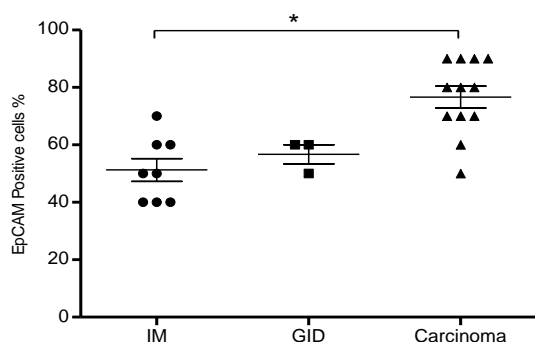


Figure 1. Average epithelial cell adhesion molecule (EpCAM) positive cells in intestinal metaplasia (IM), gastrointestinal dysplasia (GID), and carcinoma. Data were represented as mean \pm standard deviation (SD).

$P < 0.01$ IM vs. carcinoma

EpCAM expression and correlation with overall survival: Carcinoma samples were used to evaluate survival analysis. The samples with more than 75% positive cells showed a positive association with overall survival of patients (Long Rank, $P < 0.05$). The median survival time of patients with elevated EpCAM expression was 105 days in comparison with samples with lower EpCAM expression (45 Days) (Figure 2).

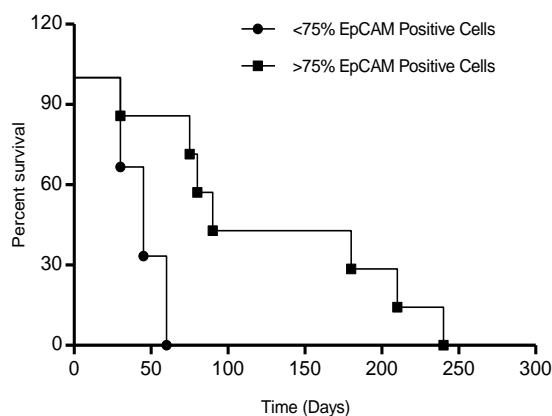


Figure 2. Kaplan-Meier survival curve: overall survival analysis according to different percentages of epithelial cell adhesion molecule (EpCAM) positive cells among patients suffering from carcinoma.

Discussion

EpCAM is an adhesion molecule on the epithelial cells that is primarily identified as a tumor marker for carcinomas.^{7,10,12} In this study, the EpCAM expression was investigated in different stages of gastric cancer in addition to the evaluation of the association of EpCAM overexpression with overall survival of patients with carcinoma. It was observed that EpCAM overexpression in all stages of gastric cancer (IM, GID, and carcinoma), while its expression in early stages, was lower than the advanced stage, and staining intensity in the advanced stage was more than the early stage. Accordingly, EpCAM has an important role in the carcinogenesis of gastric cancer. Kroepil et al. identified that in gastric cancer, EpCAM overexpression was correlated with higher tumor cell proliferation and higher lymph node metastasis.⁵ Moreover, Wenqi et al. indicated EpCAM overexpression in gastric cancer tissues and cell lines. They revealed that EpCAM down regulation leads to cell proliferation decline, cell cycle arrest in SGC7901 and human gastric adenocarcinoma cell line (AGS) cells, and blocked tumor formation in nude mice.¹ In contrast with the results of the present study, Joo et al. revealed that EpCAM expression was higher in the early stage of gastric cancer.¹³

Differences in tissue processing and the use of different primary anti-EpCAM antibodies may be the likely reasons. EpCAM expression was used for the classification of gastric cancer⁵ and as an ideal target for immunotherapy.⁶

Mukherjee et al. showed that the EpCAM was overexpressed in the tumor stroma of prostate cancer.¹⁴ Yanamoto et al. found EpCAM overexpression in squamous cell carcinoma (SCC) of the tongue and EpCAM expression was related to invasion pattern, tumor size, and regional lymph node metastasis.¹⁵ Pak et al. identified EpCAM overexpression in SCC of lung cancer.¹⁶

Conclusion

In conclusion, the current results revealed that in the early stage of gastric cancer, EpCAM expression was increased and its expression was elevated with the progress of the tumor. However, EpCAM overexpression showed lower survival in a patient with carcinoma. Nevertheless, it should be taken into account that this experiment was carried out on a limited number of patients and further studies employing a high number of patients are required.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgments

The authors wish to extend their gratitude to the Research Deputy of Kurdistan University of Medical Sciences Sanandaj, Iran, for their financial support. The financial support of this study was provided by Kurdistan University of Medical Sciences to Ali Jalili.

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