Forbes J Med 2020;1(1):90-5
doi: 10.5222/forbes.2020.43153

Özgün Araştırma / Original Article

Tissue Expression of ETS-Related Gene in Gastric Carcinomas

Mide Karsinomlarında ETS ile İlişkili Genin Doku Ekspresyonu

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Cite as: Sayhan S, Diniz G, Birol İE, Solakoglu Kahraman D, Çalik B. Tissue expression of ETS-related gene in gastric carcinomas. Forbes J Med. 2020;1(3):90-5.

ABSTRACT

Objective: The ETS-related gene (ERG) encodes a member of the erythroblast transformation-specific (ETS) transcription factor family that has been implicated in both tumor invasion and neovascularization. In this retrospective study, we have aimed to investigate the clinical significance of ERG tissue expressions in gastric cancer.
Method: We have examined the expression of ERG protein using immunohistochemical staining in tissue specimens harvested from 172 primary gastric cancer cases.
Results: ERG was expressed in normal vascular endothelium. ERG staining was defined as positive in 9.9% (17/172) of gastric cancer cases. According to clinicopathological characteristics, statistically significant differences were not observed between ERG expression and tumor invasion, lymph node and distant metastases, increased tumor stage, histological lymphatic and neural invasion. There were no significant differences in terms of age, gender, or histopathological grading as for ERG expression.
Conclusion: ERT expression in gastric cancers is not correlated with histological prognostic indicators.

Keywords: Gastric Carcinomas, ETS, ERG, prognostic criteria

ÖZ

Amaç: ETS ile ilişkili gen (ERG), hem tümör invazyonunda hem de neovaskülarizasyonda rol oynayan eritroblast transformasyona spesifik (ETS) transkripsiyon faktör ailesinin bir üyesini kodlar. Bu retrospektif çalışmada, mide kanserinde ERG doku ekspresyonlarının klinik önemini araştırmayı amaçladık.
Gereç ve Yöntem: Yüz yetmiş iki primer mide kanserinden elde edilen dokularda immünohistokimyasal boyama ile ERG proteininin ekspresyonunu inceledik.
Bulgular: ERG, normal vasküler endotelyumda eksprese edildi. Mide kanseri vakalarının %9,9'unda (17/172) ERG boyaması pozitif okolarak tanımlandı. Klinikopatolojik özellikler açısından, ERG ekspresyonu ile tümör invazyonu, lenf nodu metastazı, uzak organ metastazı, artan tümör evresi, lenfatik ve nöral invazyon arasında istatistiksel anlamlı ilişki gözlenmedi. Yaş, cinsiyet yveya histopatolojik derecelendirmede de ERG ekspresyonu açısından önemli bir farklılık yoktu
Sonuç: Mide kanserlerinde ERT ekspresyonu histolojik prognostik göstergelerle ilişkili değildir.

Anahtar kelimeler: Mide Karsinomları, ETS, ERG, rognostik kriterler

Received/Geliş: 06.10.2020
Accepted/Kabul: 30.10.2020
Publication date: 30.12.2020

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INTRODUCTION

The ETS-related gene (ERG) encodes a member of the erythroblast transformation-specific (ETS) transcription factor family.[1,2] All members of this family are key regulators of embryonic development, cell proliferation, differentiation, angiogenesis, inflammation, and apoptosis. The protein encoded by this gene is expressed in the nuclei.[1-3] This protein is necessary for platelet adhesion to the subendothelium by inducing vascular cell remodeling. In addition, it regulates hematopoiesis and differentiation and maturation of megakaryocytes.[3,4] This gene plays a role in chromosome translocations and different fusion gene products are formed as a result of TMPSSR2-ERG and NDRG1-ERG sexspressions in prostate cancer, EWS-ERG expression in Ewing sarcoma, and FUS-ERG-like translocations in acute myeloid leukemia.[5-10]

The ERG gene, located on chromosome 21, was first described in humans in 1987 by Reddy et al. in colorectal carcinoma cells.[5] ERG was first observed in the embryonic mesoderm and endothelium in the development process and is expressed in the vascular system, urogenital tract and localizations that play a role in bone development. In addition, it is highly expressed in neural crest cells during the migration phase.[1-4] ERG is thought to be oncogenic as it acts as a transcription factor regulating genes involved in tumor proliferation and invasion.[5,6]

Gastric carcinomas rank second among the most frequently seen life-threatening cancers in the world. Since at the time of diagnosis 90% of the cases are in their advanced stages, these patients have relatively lower survival rates. Interactions among individual factors such as diet, Helicobacter pylori (HP) infection, environmental factors, and genetic predisposition lead to the development of gastric carcinoma.[7] Correa's gastric carcinogenesis cascade accepted by many investigators starts with HP infection, and follows a course passing through the stages of superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia, and finally gastric carcinoma.[8,9]

Hitherto, as relevant markers for assessing the proliferative activity and tumor cell dynamics of gastric carcinomas, many parameters have been suggested. However, among these parameters ERG has not been extensively investigated.[6,10] In this study we have aimed to evaluate statistical significance of ERG expression in gastric cancers.

MATERIAL and METHODS

In this retrospective descriptive study, demographic data, and medical information including age, and gender of the patients, location, diameter and TNM stage of the tumor related to 172 cases with gastric carcinoma treated at our institution from 2011 to 2018, were retrospectively evaluated. All cases were also investigated regarding type, and grade of the tumor, lymphovascular, perineural invasion, and lymph node involvement. This study has been conducted in accordance with the principles of the Helsinki Declaration and was approved by the local Ethics Committee of Izmir Democracy University (2019/03).

The paraffin block most suitable for immunohistochemical (IHC) evaluation was selected. Firstly, the slides, and then the blocks were labeled, and cylindrical tissue samples with a diameter of 2 mm were harvested from donor blocks. Then, microarray blocks were prepared using mapping and addressing techniques.

IHC tests were performed using the streptavidin-biotin peroxidase method (Invitrogen, Camarillo, 85-9043, USA). Serial 5-µm sections were obtained from prepared microarray blocks and placed on slides which were baked overnight at 60°C, dewaxed in xylene, and hydrated with distilled water through decreasing concentrations of alcohol. All slides were treated with heat-induced epitope retrieval procedure in a microwave. In this procedure slides were left for 20 min in 10mM/L citrate buffer at pH 6.0, cooled at room temperature for 20 minutes, and then blocked to retrieve endogenous peroxidase and biotin. Purified monoclonal antibodies against ERG (Ventana, Basel, Swiss, clone EPR3864, ready-to-use kit) were used. Nuclear expression of ERG in

endothelial cells in tumor tissue samples was accepted as an internal control (Figure 1). The pathologist who was blinded to the clinical features of the patients examined the slides and staining patterns were classified according to the intensity or presence of staining. Strong nuclear staining in tumor cells was evaluated as positive ERG expression. Quantitative evaluation could not be made because the staining pattern was focal and not homogeneous.

Statistical analysis was performed using statistical package of SPSS 25.0. For the comparison of the quantitative data chi-square test was used. For the comparison of parametric data, independent samples t-test, and for nonparametric data Mann-Whitney U test were used. For the comparison of the measurements in more than 2 groups nonparametric Kruskal-Wallis test was employed. P≤0.05 was accepted as the level of statistical significance.

RESULTS

In this study 172 patients gastrectomized with the indication of gastric carcinoma were evaluated. Fifty-seven (33.1%) patients were female and 115 patients (66.9%) were male. The mean age of patients was 64±12.3 years (between 29 and 92 years). The patients were followed up for a mean period of 25.3± 22.8 months. Gastric carcinomas were localized on cardia in 37 (21.5%), corpus in 75 (43.6%), and antrum/pylorus in 60 (34.8%) cases. Mean diameter of the tumors was 5.8±3.2 cm (range: 1-15 cm). Their histopathological subtypes consisted of poorly cohesive (n=57), tubular (n=90), and mixed type (n=25) carcinomas. Local lymph node metastasis were detected in 132 (76.7%) cases. Number of metastatic lymph nodes ranged between 1, and 44 (mean: 6.6± 8.7) nodes. Based on TNM classification, the cases were evaluated in categories of T4 (n=59), T3 (n=79), T2 (n=24), T1 (n=10), N0 (n=34), N1 (n=36), N2 (n=41), N3a (n=38), and N3b (n=23). Distant organ metastases were observed in 41 (23.8%) cases. Metastases were localized in liver (n=18), lungs (n=13), peritoneum (n=8), and ovaries (n=2).

Twenty-nine cases (16.9%) were evaluated as HER2-positive using IHC and FISH methods in combination, and all of these cases received targeted therapy. HER2- positive tumors were localized in gastric cardia (n=5), corpus (n=19) or pylorus (n=5). A statistically significant relationship was not found between HER2 status and tumor localization (p:0.539). Metastatic cases were more numerous in HER2-positive group (61.1%) compared to the HER2 negative group (25.6%). A statistically significant difference was detected between HER2-positivity and metastases (p=0.042). Similarly, the rate of mortality in the HER2-positive group (65.5%) was slightly higher than the HER2-negative group (52.4%). However, there was no statistically significant difference between HER2 positivity, mortality rates (p=0.197) and survival time (p=0.671).

The average ages of the patients with HER2-positive, and HER2-negative tumors were 63.1, and 64.2 years, respectively. The average tumor diameters of HER2-positive and negative tumors were 6.6 cm, and 5.7 cm, respectively. In summary, there was no statistically significant relati-

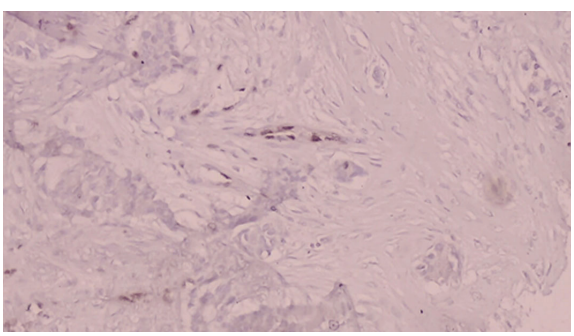

Figure 1. ERG expression observed only in endothelial cells in tumor stroma (DABx 400).

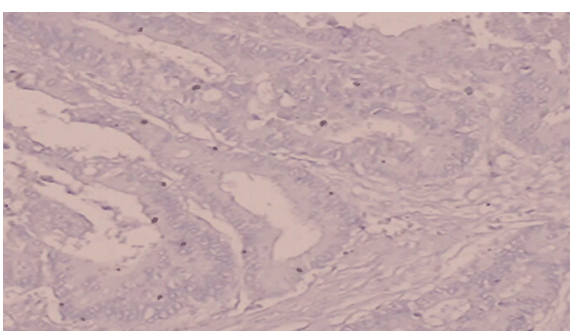

Figure 2. Nuclear ERG expression in a tissue sample of tubular gastric carcinoma (DABx 200).

onship between HER2 status and age (p:0.670), tumor size (p:0.199), lymph node metastasis (p:0.875), and tumor stage (p:0.763).

In only 17 cases (9.9%) nuclear expressions of ERG were detected in tumor cells (Figure 2). Any significant relationship did not exist between ERG expression and survival rates (p=0,257), age (p:0.943), tumor size (p: 0.378), tumor location (p:0.244), lymph node metastasis (p:0.575), HER2 status (p:0.574), and tumor stage (p:0.903).

DISCUSSION

Studies have shown that ETS proteins are nuclear DNA-binding phosphoproteins that act as activators of transcription suppressors.[1-4] Twenty-eight out of 30 genes in the ETS family have been identified in the human genome. ERG, known as the oncogene associated with this family which has the potential to form new oncogenic proteins as a result of some fusions in different tumors.[1-5] Today, one of the most studied tumors associated with ERG is prostate adenocarcinoma. In more than half of prostate adenocarcinomas, there is fusion between ERG and the androgen-regulating gene transmembrane protease, serine 2 (TMPSSR2).[6,11] ERG fusion protein is thought to stop maturation in early stem cells in the prostate and initiate carcinogenesis. ERG fusion prostate adenocarcinoma was first reported in 2005 by Tomlins et al. and it has been indicated in the literature that this fusion is seen up to 60 % of the the cases with prostate carcinoma, especially in western societies.[6,11-14] In our study, we found ERG expression only in 9.9% of the gastric carcinomas. There was no evidence indicating that ERG expression contributes to the proliferative capacity of gastric carcinomas.

It is seen that the rates of ERG expression found in the literature vary widely, especially in cases with prostate carcinoma. In different studies, immunohistochemical ERG-positivity has been reported between 10% and 68% in prostate adenocarcinoma cases.[11-14] Although ERG expression is generally generally examined immunohistochemically in studies, some researchers have used methods such as FISH and PCR.[11-14] This variability in ERG expression rates can be thought to be due to the selection of different ERG detection methods. However, a strong correlation has been reported between immunohistochemical methods and FISH used in the detection of ERG expression in prostate tissue in terms of sensitivity and specificity.[11] Tumor heterogeneity may also be important in the detection of varying ERG expression rates over a wide range. In our study, ERG expression was detected only in a few tumor specimens. Considering that ERG expression may be heterogeneous, performing ERG examination on different sections taken from different tumor areas may increase the rate of ERG- positivity in gastric carcinomas.

Variations in the expressions of ETS/ERG gene are also important in the etiopathogenesis of Ewing sarcoma (ES). Extraskeletal ES usually originates in the deep soft tissues of the extremity, paravertebral, retroperitoneal, mediastinal, head and neck, and also thoracopulmonary (Askin tumor) regions. Symptoms such as pain, swelling, fever, weight loss, and indolence, and sensory or motor disorders such as paralysis, incontinence, and numbness in the neighborhood of the spinal region can be observed. EWSR1-FLI1 and EWSR1-ERG translocations, where the EWSR1 gene localized on the chromosome 22 and the ETS gene family join, is important in its pathogenesis. These translocations are the original diagnostic findings in differentiating ES from other round cell malignant tumors.[15,16] However, ES was not considered in the differential diagnosis of gastric carcinomas.

ERG, a gene defined at the most common breakpoint on chromosome 21 in AML, encodes a protein that has a regulatory role in the lower steps of the mitogenic signaling pathway. In AML, FUS/ERG fusion resulting from t (16; 21) (p11; q22) is associated with a poor prognosis.[17] In addition, it has been shown that overexpression of ERG is a risk factor in adult patients with ALL.[18] Most hematological malignancies are very different from gastric carcinomas in their clinical course and rarely fall under the scope of cancers that require differential diagnosis. Since only

myeloid sarcoma forms a solid tumor, clinical overlap may exist.[19]

ERG expression can be reliably determined by immunohistochemical methods. Since there is no ERG expression in benign prostate tissue and stromal cells, detection of ERG- positivity supports the diagnosis of prostate carcinoma. ERG expression in normal prostate tissue can only be observed in endothelial cells. ERG can also be evaluated using fluorescence in situ hybridization (FISH) method or PCR. It is reported in the literature that there is no difference between FISH and IHC in terms of detecting ERG expression.[13]

This study is one of the rare studies concerning ERG expression in gastric carcinomas. Although the relationship between ERG expression and prostate cancer has been established, ERG expression in gastric cancers has not been evaluated before. In our study, very low levels of ERG expression were detected in gastric carcinomas, and there was no relationship between prognostic factors. In the present study, we hoped that ERG may be used to predict the prognosis of gastric cancers. However, ERG could not predict the behavior of gastric tumors.

Ethics Committee Approval: The study protocol was approved by the Local Ethics Committee of Izmir Democracy University (2019/03).

Conflict of Interest: No conflict of interest has been declared by the authors.

Funding: The authors of this research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Informed Consent: As the study was retrospective, consent was not obtained from the patients.

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