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MUTATION STATUS AND IMMUNOHISTOCHEMICAL CORRELATION OF *EGFR* MUTATIONS IN GASTROINTESTINAL STROMAL TUMORS

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ABSTRACT

Being one of the leading causes of cancer deaths worldwide and their resistance to conventional treatment methods, made gastrointestinal stromal tumors (GISTs) one of the hot topics in medical research areas in the past decade. To investigate molecular alterations underlying the tumor is of great importance to be able to develop new, targeted treatment options. In this study, GIST samples obtained from 40 Turkish patients were analyzed for actionable epidermal growth factor receptor (*EGFR*) mutations that are related to treatment regimes in non small cell lung cancer (NSCLC) to understand whether *EGFR* expression is altered in GISTs. Established alterations in *EGFR* can make the use of tyrosine kinase inhibitors possible, which are currently used in cancer therapy, especially in NSCLC. Our results indicated that *EGFR* mutations are rare in GISTs. Further research is needed to sequence whole coding regions of the gene to investigate new actionable mutations in *EGFR* in an increased sample size.

Keywords: Epidermal growth factor receptor (*EGFR*) gene; Gastrointestinal stromal tumors (GISTs); Targeted therapy.

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal (GI) tract, generally occurring after the age of 50. A majority of all GISTs occur in the stomach (~60.0%), followed by the jejenum/ileum, duodenum, rectum, appendix, colon and rarely, in the oesophagus [1]. Aggressive GISTs can me-tastasize to the liver and throughout the abdomen [2]. Patients frequently develop abdominal pain, nausea, fatigue and GI bleeding as the symptoms of GIST, while some remain asymptomatic [3]. Gastrointestinal stromal tumors are one of the leading causes of cancer deaths worldwide, therefore, understanding its molecular background and developing targeted therapy techniques are of high importance [4,5].

At the cellular level, GISTs are known to have a broad morphological spectrum. In general, they are divided into three histological subtypes: the spindle cell type being the most common, epithelioid type and rarely mixed spindle cell and epithelioid type [6]. In the last decade, investigation of alterations at the molecular level underlying the disease, greatly revolutionized both diagnosis and treatment strategies. The vast majority of GISTs were found to have activating mutations in the KIT receptor tyrosine kinase, which is accepted as a GIST biomarker by the European Group on Tumor Markers (EGTM). Currently, detection of CD117 (c-KIT) by immunohistochemistry is the method used for

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diagnosis or confirmation of imaging-based diagnoses [7]. Less frequently, mutations in the gene platelet-derived growth factor receptor α (PDGFRA) are detected in the KIT-negative GISTs [8-11]. On the other hand, expression or function of another well-known receptor tyrosine kinase, the epidermal growth factor receptor (*EGFR*) gene was found to be frequently altered in colorectal and gastric cancers as in many other tumor types [12,13]. The *EGFR* family comprises *ERBB2/HER2, ERBB3/HER3* and *ERBB4/HER4* as well as the *EGFR* itself [14]. Binding of a ligand causes dimerization of receptors that leads to autophosphorylation of tyrosine residues, which then phosphorylates downstream signaling molecules triggering cellular pathways involved in DNA synthesis, cell growth, proliferation and differentiation [15].

Before the investigation of such molecular alterations and development of targeted treatments, complete surgical resection was the only potentially curative treatment of choice for localized GISTs [3]. However, use of tyrosine kinase inhibitors (TKIs) and monoclonal antibodies (mAbs) as targeted therapy agents to inhibit carcinogenic actions of the tyrosine kinases in various human cancers, was shown to prolong overall survival and progression-free survival in cancers including GISTs, breast cancer, non-small cell lung cancer (NSCLC), and pancreatic cancers in the last decade [16].

Therefore, the importance of being able to detect mutations in cancer patients causing the disease and prescribing a therapy accordingly is clear. To the best of our knowledge, even though it is studied extensively in many tumor types, frequency of somatic *EGFR* mutations in GISTs has not been studied in the Turkish population before. In this study, mutation status of the *EGFR* gene and immu-nohistochemical marker changes in GISTs from 40 different Turkish patients was investigated to compare and correlate the mutation status with histopathological changes in the patient samples.

MATERIALS AND METHODS

Study Design, Participants and Immunohistochemical Analysis. In this study, 40 patients' samples (19 females, 21 males) who were diagnosed with GIST, were found to be positive with c-KIT immunohistochemistry staining (Clone YR145; Cell Marque Corporation, Rocklin, CA, USA) and DOG-1 (Clone K9; Leica Biosystems, Wetzlar, Germany) between January 2013 and June 2018, at the Pathology Department of Şişli Hamidiye Etfal Education and Research Hospital, Istanbul, Turkey.

All 40 tumor samples were fixed in 10.0% formalin for 10 hours at room temperature. Then they were embedded in paraffin sections (4 μm thick) and mounted onto positively-charged glass slides. Immunostaining was performed with an automated immunostainer (Ventana Medical Systems, Inc., Tucson, AZ, USA) using the *EGFR* antibody (Clone: EGFR.113, dilution: 1/200, Novocastra Laboratories Ltd., Newcastle, Tyne and Wear, UK) at the Pathology Department of Near East University Hospital, Nicosia, Cyprus. The stained slides were evaluated semi-quantitatively by two independent pathologists. The *EGFR* scoring system was referred from the study of Edris *et al.* [17] as 0: absence of any staining; 1: weak staining (diffuse or focal); 2: strong staining (diffuse or focal).

Genetic Analysis. DNA was isolated from the tumor tissue embedded in paraffin blocks using QIAamp® DNA FFPE Tissue Kit (Cat.: 56404, Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. Then, Therascreen® EGFR Pyro Kit (Cat.: 971480, Qiagen GmbH) was used for sequence-based detection and quantitation of mutations in the exons 18 (codon 719), exon 19 (deletion), exon 20 (codons 768 and 790) and exon 21 (codons 858 and 861) of the *EGFR* gene, according to the manufacturer's protocol, using 10 ng of genomic DNA extracted from the tumor tissue at the Department of Medical Genetics, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey.

RESULTS

Nineteen female and 21 male patients (total 40) participated in the study with average ages 57.3 and 62.4, respectively. Minimum and maximum ages in women were 24 and 78, whereas it was 45 and 77 in men. Age and gender of the patients, location and cell types of tumors are shown in Table 1 below.

At the end of the molecular genetic analysis, no mutations were detected in the most common six hot-spot regions that included codon 719 in exon 18, deletion of exon 19, codons 768 and 790 in exon 20 and codons 858 and 861 in exon 21 of the *EGFR* gene tested in 40 different somatic GIST patients. Probably, immunohistochemical analyses showed no expression of EGFR in the tested somatic tumor samples (Figure 1).

DISCUSSION

Gastrointestinal stromal tumors gained particular interest over the last decade as they are the most common mes-enchymal neoplasm in the GI tract, accounting for ~1.0% of all GI tumors, and are resistant to conventional chemotherapy and radiotherapy options [6,18]. In the era of precision medicine, discovery of particular molecular aberrations in GISTs promised novel treatment options applicable to the patients. Most GISTs were found to have

Table 1. Patient details such as age, gender, tumor locations, cell types and *EGFR* gene mutation status are shown.

Sex	Mean Age	Tumor Location (%)	Tumor Cell Type (%)	Status of Analyzed *EGFR* Gene Mutations Exons (codons)				*EGFR* Expression
				18 (719)	19 (deletion)	20 (768; 790)	21 (858; 861)	
F	57.2±5.6	duodenum (21.5)	spindle cell (100.0)	wild type	wild type	wild type	wild type	negative
		ileum (10.5)	epithelioid (100.0)	wild type	wild type	wild type	wild type	negative
		small intestine (26.3);	epithelioid (20.0); mixed (60.0); spindle cell (20.0)	wild type	wild type	wild type	wild type	negative
		stomach (38.8)	spindle cell (57.0); mixed (14.2); epithelioid (28.8)	wild type	wild type	wild type	wild type	negative
		transverse colon (5.2)	mixed (100.0)	wild type	wild type	wild type	wild type	negative
M	62.6±10.5	colon (9.5)	spindle cell (100.0)	wild type	wild type	wild type	wild type	negative
		descending colon (4.7)	mixed (100.0)	wild type	wild type	wild type	wild type	negative
		duodenum (4.7)	spindle cell (100.0)	wild type	wild type	wild type	wild type	negative
		ileum (4.7)	mixed (100.0)	wild type	wild type	wild type	wild type	negative
		jejenum (4.7)	spindle cell (100.0)	wild type	wild type	wild type	wild type	negative
		small intestine (19.0)	spindle cell (25.0); mixed (75.0)	wild type	wild type	wild type	wild type	negative
		Stomach (52.4)	spindle cell (54.5); mixed (36.3); epithelioid (9.2)	wild type	wild type	wild type	wild type	negative

EGFR: epidermal growth factor receptor; F: females; M: males.

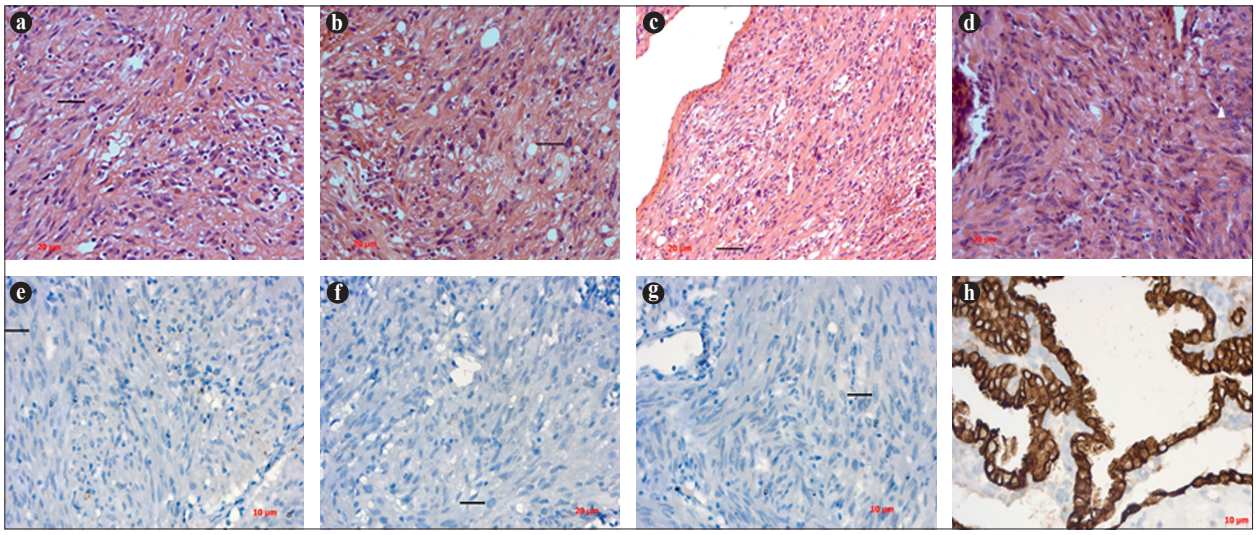

Figure 1. Images **a-d** show immunohistochemical staining of GIST samples with spindle-shaped tumor cells, negative for *EGFR* expression. Images **e-f** indicate hematoxylin and eosin staining of GIST samples with spindle-shaped tumor cells. Image **g** represents the positive control, expressing *EGFR*. All images were taken in 40× magnification. Image **h** represents the control *EGFR* sample from a chorionic villus sample.

activating mutations in two closely related tyrosine kinase receptors, *KIT* and *PDGFRA*. When they are mutated, the receptors become constitutively active and trigger uncontrolled cell proliferation leading to tumor formation. Therefore, use of specific tyrosine kinase inhibitors such as imatinib mesylate, restores normal signaling and was proved useful in the treatment of GISTs [18]. Discovery of such molecular markers and targeted treatments help to reduce the time consumed for diagnosis and decision of treatment method, improving survival times of patients.

In this context, different alterations in the *EGFR* gene have also been used as a molecular marker for various tumor types, allowing the use of kinase inhibitors as effective treatment strategies in patients. The *EGFR* gene can gain oncogenic activity through structural rearrangements, gene amplifications and activating point mutations [19]. Point mutations generally cluster in the region that codes for the tyrosine kinase domain (exons 18-21) of the receptor, which results in constitutive activation of the encoded *EGFR* even in the absence of its ligand, resulting in excessive cell growth and proliferation leading to tumorigenesis [12].

In colorectal cancers, the *EGFR* gene copy number was shown to be high compared to normal tissue, somatic mutations affecting the kinase domain of the protein was seen frequently in NSCLC [20]. In gastric cancers, overexpression of the gene was well described, however, clinical trials targeting EGFR mostly returned disappointing results, probably because the patient selection procedure was not biomarker-assisted [12,21]. Additionally, aberrations in *EGFR* are frequent in other tumors including breast, brain and ovary. Use of anti-EGFR monoclonal antibodies or EGFR-targeted tyrosine kinase inhibitors is proven to be successful in these tumors. In addition to its specificity, TKIs in general are administrated orally and provide a rapid tumor response, unlike conventional cytotoxic chemotherapy options [22].

In this study, *EGFR* status of 40 somatic GIST samples derived from stromal mesenchymal origins were analyzed to understand whether any *EGFR* aberrations are present in GISTs to be potentially used in diagnosis and treatment of these tumors. Despite previous studies [20,22,23] that showed no significant association between EGFR expression and prognostic analysis of GISTs, a study by Shi *et al.* [24] indicated that only a small percentage of GISTs carry somatic *EGFR* mutations but speculated that it may play a role in the development and progression of the GISTs. However, the literature about the EGFR status in GISTs is still very limited. In the present study. The GIST samples were tested for therapy-targeted somatic *EGFR* mutations that are found in many cancer types (such as lung, breast, *etc.*) in the kinase domain region by targeted sequencing, and immunohistochemistry was also used for detection of any overexpression of the *EGFR* at the protein level. Data analyses indicated no mutations and no overexpression in the samples tested. The results explain that EGFR mutations potentially left out from primarily GISTs tumors. Therefore, these EGFR mutation-free GISTs have likely been resistant to TKI therapies. On the other hand, Apicella *et al.* [25] indicated that EGFR cannot be totally ignored as a potential target in gastric cancer, the EGFR pathway function should be examined for each subject considering the inhibition of the EGFR. According to another study [26], a phosphorylation of the EGFR pY1068 type was observed in the chromosomal instability as well as *EGFR* mutations, which vascular endothelial growth factor receptor 2 (VEGFR) targeted antibodies were recommended to gastric cancer patients.

The main limitation of our study was the sample size. The study requires more patient samples and clinical data to support somatic *EGFR* mutations as serving as a prognostic biomarker for clinical decision making in GISTs. Moreover, we have only examined known driver mutations, which respond to treatment in other cancer types such as lung cancer. In the future study, full coding gene region sequencing analysis of the EGFR gene can be designed.

Overall, supporting the previous study by Shi *et al.* [24], our results indicate that somatic *EGFR* mutations are rare in GISTs. Despite a bigger sample size being needed to confirm this conclusion [27], these primary data support that EGFR-tyrosine kinase inhibitor (TKI) treatment alone may not have impact on patients' survival. However, it should be further investigated whether *EGFR* has a role in the initiation of these tumors.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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