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Gastric GISTs: Analysis of *c-Kit*, *PDGFRA* and *BRAF* mutations in relation to prognosis and clinical pathological characteristics of patients – A GIRCG study

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Abstract

Background: Gastric gastrointestinal stromal tumors (GISTs) represent a subgroup of GISTs with a better prognosis than those located in other areas. In this retrospective study we performed a molecular characterization of a large series of patients with gastric GISTs in relation to clinical–pathological characteristics and prognosis.
Methods: DNA was extracted from paraffin-embedded sections from 221 gastric GIST patients submitted to surgery. Exons 9, 11, 13 and 17 of *KIT*, exons 12 and 18 of *PDGFRA* and exons 11 and 15 of *BRAF* were analyzed by direct sequencing. Cox regression analysis adjusted for clinical–pathological factors was performed to evaluate *KIT* and *PDGFRA* mutations in relation to the composite endpoint of relapse or death.
Results: *KIT and PDGFRA* mutations were observed in 119 (53.8%) and 56 (25.3%) patients, respectively, whereas 46 (20.8%) patients had wild type (wt) disease. Univariable analyses showed that a high Miettinen risk category and the presence of ulceration and *KIT* deletions

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were associated with increased risk of relapse or death ($p < 0.001$; $p = 0.0389$ and $p = 0.002$, respectively). After adjusting for Miettinen risk score, *KIT* deletions remained an independent prognostic factor ($HR_{adj} = 2.65$, 95% CI [1.15–6.13], $p = 0.023$). Moreover, *KIT* deletions in exon 11 codons 557, 558 or 559 were associated with a higher risk of relapse or death than wt tumors ($HR_{adj} = 3.29$ 95% CI [1.64–6.64], $p = 0.001$).

Conclusions: *KIT* deletions in exon 11, especially those involving codons 557, 558 or 559, were correlated with a more aggressive gastric GIST phenotype and increased risk of relapse or death.

Keywords: GISTs; *KIT*; *PDGFRA*; *BRAF*; Prognostic factors; Mutation

Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract and can develop in any part of this area; 60–70% of clinically manifest tumors arise in the stomach and 20–30% in the small intestine, but a small percentage also occurs in the rectum, colon, esophagus or omentum.[1–3] About 70–80% of GISTs harbor a *KIT* gene mutation in exons 11 (about 60% of cases) and 9 (7–10%) and, less frequently, in exons 13 and 17.[4–6] About 20–30% of *KIT* wild type (wt) GISTs show mutations in the platelet-derived growth factor receptor alpha (*PDGFRA*) gene, in particular in exons 12, 14 and 18,[6,7] while a lower percentage (4–13%) have *BRAF* mutations.[8–10]

Gastric GISTs represent a subgroup of GISTs with a favorable prognosis and are characterized by a relatively higher fraction of cases with an epithelioid or mixed epithelioid/spindle morphology, a higher frequency of *PDGFRA* mutations and a lower frequency of *KIT* alterations, a lower mitotic index and overall lower mortality than other GISTs.[11–14] Whilst the predictive role of *KIT* and *PDGFRA* mutations in relation to response to imatinib is well known,[15–17] the prognostic significance of these mutations and of the type of mutation has yet to be defined. Some studies have shown that gastric GISTs with exon 11 deletions have a worse outcome than those with single nucleotide substitutions at the same exon.[18–20] *PDGFRA* exon 18 mutations have also been associated with a lower risk of metastasis and a better prognosis.[12,18] Other studies have demonstrated that, in addition to different exons, the type of mutation and several codons affected by mutations may have different prognostic implications. In particular, *KIT* exon 11 deletions are associated with a risk of metastasis, while those involving codons 557–558 indicate a higher risk of progression.[21–25] Conversely, single *KIT* exon 11 substitutions have been correlated with longer relapse-free and overall survival.[15,21,23,25,26] Moreover, *KIT* exon 9 duplications, which occur mainly in intestinal tumors, have been associated with aggressive behavior.[21,25]

A number of studies have also analyzed *BRAF* gene alterations in GISTs, reporting a mutation frequency of about 4–13%.[8–10] A predominant small intestinal location of GISTs with *BRAF* V600E has been observed, followed by a location in the stomach.[8] *BRAF* mutations are not *per se* indicative of malignancy in that they have not been found to show a significant correlation with prognosis.[9] However, a recent study in which GIST patients were divided into 3 prognostic groups on the basis of type of mutation found that *BRAF* mutations were associated with the group with the best prognosis, suggesting a positive prognostic effect of this alteration.[27]

The main aim of our retrospective study was to assess *KIT*, *PDGFRA* and *BRAF* mutations in a large series of patients with gastric GISTs recruited by member centers of the Italian Research Group of Gastric Cancer (GIRCG). We analyzed different gene mutations and types of mutation in relation to the clinical–pathological characteristics of patients to see whether this information could be used to improve the clinical management of the disease.

Materials and methods

Case series

We retrospectively analyzed a cohort of 221 patients with gastric GISTs submitted to surgical resection between March 1985 and December 2012. All cases were recruited from 8 member centers of the Italian Gastric Cancer Research Group (GIRCG). Information on clinical–pathological data such as tumor size, mitosis, presence of ulceration, necrosis, atypia and type of cellularity was collected by reviewing all available medical and histopathological records archived in GIRCG centers. The study was approved by the Local Ethics Committee of each center.

Histopathological variables analyzed for each tumor were as follows: size, mitotic count per 50 high-power fields (HPF), cell type, presence or absence of ulceration, necrosis and nuclear atypia, and pattern of *KIT* and *MIB1* immunostaining. Immunohistochemical staining was performed using the following primary antibodies: KIT (CD117 antigen, Dako Corporation, Carpenteria, CA, USA) and Ki67 (MIB1, Dako Corporation).

On the basis of the Miettinen risk score, GISTs were stratified as no-, very low-, low-, intermediate- and high-risk tumors.[2]