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Original contribution

Chromosomal aberrations in primary *PDGFRA*-mutated gastrointestinal stromal tumors☆

Inga-Marie Schaefer MD[a,*], Christian Delfs MD[a], Silke Cameron MD[b], Bastian Gunawan MD[a], Abbas Agaimy MD[c], B. Michael Ghadimi MD[d], Florian Haller MD[c]

[a]*Department of Pathology, University Medical Center Göttingen, Robert-Koch-Straße 40, Göttingen D-37075, Germany*
[b]*Department of Gastroenterology and Endocrinology, University Medical Center Göttingen, Robert-Koch-Straße 40, Göttingen D-37075, Germany*
[c]*Institute of Pathology, University Medical Center Erlangen, Krankenhausstraße 8-10, Erlangen D-91054, Germany*
[d]*Department of General and Visceral Surgery, University Medical Center Göttingen, Robert-Koch-Straße 40, Göttingen D-37075, Germany*

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Summary Approximately 15% of gastrointestinal stromal tumors (GISTs) harbor mutations in the platelet-derived growth factor receptor α (*PDGFRA*) gene. Chromosomal aberrations play a crucial role in tumor progression and correlate with clinical behavior. Imbalances, particularly in *PDGFRA*-mutated GISTs, have not yet been evaluated in larger series. We analyzed 53 *PDGFRA*-mutated GISTs (including 2 with corresponding metastases) for chromosomal imbalances by conventional comparative genomic hybridization and compared them with a historical collective of 122 *KIT*-mutated GISTs. *PDGFRA* exon 18 mutations (91% of cases) and exon 12 mutations (9% of cases) correlated significantly with gastric and intestinal sites, respectively. The most common aberrations were identical to those found in *KIT*-mutated GISTs, with −14q in 70%, −1p in 28%, and −22q in 17% of cases. Overall, there were significantly fewer chromosomal aberrations compared with *KIT*-mutated GISTs, with a mean of 2.8 (0.6 gains, 2.1 losses) aberrations per tumor. There was a statistically significant association of more than 5 chromosomal imbalances with intermediate/high-risk categories. Regarding specific chromosomal aberrations, −9p, −13q, and −22q correlated with intermediate/high risk, and −1p and +8q with poorer survival, although progression occurred in only 2 cases. Altogether, *PDGFRA*-mutated GISTs display the same chromosomal aberrations as *KIT*-mutated GISTs, although they have a lower degree of chromosomal instability in line with their generally favorable outcome.

1. Introduction

Gastrointestinal stromal tumors (GISTs) originate at any site throughout the gastrointestinal tract from the esophagus to the anorectum [1]. GISTs develop from or differentiate similar to the interstitial cells of Cajal or their precursor cells located in the gut wall [1]. They occur at an incidence of 10 to 20 per

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* Corresponding author.
E-mail address: inga-marie.schaefer@med.uni-goettingen.de (I. -M. Schaefer).

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million annually and at a median age of 55 to 65 years, affecting men and women equally [1-3]. Most GISTs occur in the stomach (60%), followed by the small intestine (30%), rectum, esophagus, omentum, and mesentery (5%-10%) [3].

Histologically, the tumor either displays a spindle cell (70%), epithelioid (20%), or mixed cell type [1]. Tumor size, anatomical site and mitotic count are relevant prognostic factors and are used to predict the risk of progression according to the Miettinen and Lasota [2] classification. Immunohistochemically, GISTs express CD117 (KIT) and CD34 in most cases (>95%, 95%, and 70%, respectively) [1,4].

On a molecular basis, activating mutations of either the *KIT* receptor tyrosine kinase (~70%) or platelet-derived growth factor receptor α (*PDGFRA*; ~10%-15%) gene are known to play a crucial role in tumor development and progression and serve as potential therapeutic targets for therapy with tyrosine kinase inhibitors [3,5-9]. *KIT* mutations affect exons 11 (66.1%), 9 (13%), 13 (1.2%), and 17 (0.6%) [1]. *PDGFRA* mutations, on the other hand, are found mainly in exons 18 (89.6%), 12 (9.3%), and 14 (1.2%) [1]. To date, no *KIT* and *PDGFRA* mutations have been described in a single GIST at the same time, indicating that these mutations are mutually exclusive [9].

PDGFRA-mutated GISTs are characterized by a weak or negative immunohistochemical staining for CD117, a low mitotic rate, an epithelioid or mixed-type morphology, and almost exclusively gastric localization [2,9-11]. However, a few cases of *PDGFRA*-mutated GISTs of intestinal origin do occur [7,12,13]. Compared with *KIT*-mutated GISTs, *PDGFRA* mutants generally follow a less aggressive clinical course with a more favorable prognosis [2,14-16].

Comparative genomic hybridization (CGH) may be used in tumors to reveal chromosomal imbalances and identify nonrandom cytogenetic events [17,18]. In GISTs lacking CD117 expression and *KIT* or *PDGFRA* mutations (ie, wild-type GISTs), the detection of characteristic aberrations by CGH may even be of diagnostic value [19]. In *KIT*- and *PDGFRA*-mutated GISTs, chromosomal losses are generally more common than gains, and frequent imbalances are −1p, −9p, −13q, −14q, −15q, −22q, and +8q [16,17,20-23].

Losses at 14q have been shown to be characteristic of gastric tumors with predominantly stable karyotypes and a more favorable clinical course, whereas the −1p and −15q pathways seem to be characteristic of intestinal GISTs with an increased capacity for cytogenetic complexity and a more aggressive clinical course previously referred to as the −1p pathway [16,17,20-23]. Independent of site, −22q may initiate the critical progression to an unfavorable pathway to increasing cytogenetic complexity including +8q, −9p, and −9q and an unfavorable clinical course previously referred to as the −22q pathway [16,17,20-23]. A previous array CGH study in 66 GISTs by Wozniak et al [16] included 18 *PDGFRA*-mutated cases and showed that genetic imbalances were equally frequent in *KIT*- and *PDGFRA*-mutated GISTs.

However, the impact of the rarer primary *PDGFRA* mutational status on cytogenetic evolution and progression of GISTs has not been studied so far. To our knowledge, this is the first analysis of chromosomal aberrations on a large cohort of *PDGFRA*-mutated GISTs (53 patients, including 2 with corresponding metastases) by using CGH to detect the most common recurring chromosomal aberrations in this subset of GISTs and present the clinicopathologic data and results of mutation analysis and CGH and compare these findings with a historical collective of 122 *KIT*-mutated GISTs to define novel diagnostic and prognostic implications.

2. Materials and methods

2.1. Patients and tumor specimens

A cohort of 53 primary GISTs with *PDGFRA* mutation, as well as 2 resected liver metastases, which were diagnosed at the Institute of Pathology at the University Medical Center Göttingen between 1993 and 2008, was included in this study. Formalin-fixed and paraffin-embedded tumor samples from each patient were examined histopathologically. Assessment of maximal tumor size, localization, histologic growth pattern, and mitotic counts per 50 high-power fields (HPFs; 0.238 mm^2) was performed independent of clinical variables. The malignancy potential was estimated based on tumor size, mitotic count, and location, according to the Armed Forces Institute of Pathology criteria published in 2006 by Miettinen and Lasota [2]. Of the 53 *PDGFRA*-mutated cases here demonstrated, 2 cases [20], 3 cases [24], and 16 cases [17] have been included in previous studies by our own group. Furthermore, a historical collective of 122 *KIT*-mutated GISTs of all sites included in a previous study [17] with known CGH status was used for comparison.

2.2. Mutation analysis

Mutation analysis of *KIT* exons 9, 11, 13, and 17 as well as *PDGFRA* exons 12, 14, and 18 was performed using direct sequencing of polymerase chain reaction products, as described previously [11].

2.3. Comparative genomic hybridization

Conventional CGH from formalin-fixed and paraffin-embedded tumor tissue specimens was performed essentially as described previously [20]. DNA was isolated from paraffin-embedded tissue specimens using a QIAamp Minikit (Qiagen, Hilden, Germany). Tumor and reference DNA were then labeled with nick translation and hybridized together with Cot-1DNA, 12 mL sodium acetate (pH 4.8; 3 mol/L), and 580 mL 100% ethanol for 3 days on normal metaphases.