**KIT and PDGFRA mutations and PDGFRA immunostaining in gastrointestinal stromal tumors**

ANTONELLA BARRECA¹, ALESSANDRO FORNARI¹, LISA BONELLO², FABRIZIO TONDAT², LUIGI CHIUSA¹, PATRIZIA LISTA³ and ACHILLE PICHI

¹Department of Biomedical Sciences and Human Oncology, Section of Pathology, University of Turin; ²Center for Experimental Research and Medical Studies (CERMS); ³Division of Medical Oncology, A.O.U. San Giovanni Battista, Turin, Italy

Received July 8, 2010; Accepted October 11, 2010

DOI: 10.3892/mmr.2010.399

**Abstract.** In the present study, we investigated the association of PDGFRA and KIT mutations as well as PDGFRA immunohistochemical expression with clinicopathologic features and prognosis in a series of gastrointestinal stromal tumors (GISTs). Tumor DNA from 40 GISTs was sequenced for the presence of mutations in KIT exons 9, 11, 13 and 17, and in PDGFRA exons 12 and 18. Tissue sections were stained with polyclonal anti-PDGFRA antibody. KIT mutations occurred in 26 cases. There were 13 deletions, 6 substitutions, 3 deletion-substitutions, 3 duplications and 1 insertion. Tumors with KIT deletions/insertion were large with a high mitotic index (MI), and were associated with a high rate of symptoms at diagnosis, invasion into adjacent organs, distant metastasis, relapse and a short disease-free survival (DFS). PDGFRA mutations occurred in 6 gastric GISTs. There were 4 deletions and 2 substitutions. Tumors with PDGFRA mutations were small, with a low MI and Ki67 score, and were associated with a very low rate of symptoms at diagnosis, invasion into adjacent organs and distant metastasis. PDGFRA immunopositivity was found in 23 cases; a peculiar ‘dotlike’ staining was found in 5 out of 6 PDGFRA mutated cases. Patients with positive PDGFRA immunostaining had a longer DFS than those with negative staining. Our data confirm that the type of KIT mutation is associated with various clinicopathologic features of GISTs, and indicate that PDGFRA mutations are associated with rather indolent tumors. PDGFRA immunopositivity reflects PDGFRA mutational status and is associated with a favorable outcome.

**Introduction**

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. Most GISTs show KIT or platelet-derived growth factor receptor-α (PDGFRA) gain-of-function mutations (oncogenic mutations) (1-4). Several studies have shown that the type of KIT mutation may be correlated with the clinical outcome of patients with GIST. A poor prognosis and metastatic behavior were found for GISTs with KIT deletions (5-13) or insertions (5,11,13). By contrast, a better prognosis was found for GISTs with KIT substitutions (point mutations) (5,10-12), deletion-substitutions or duplications (12,14). However, conflicting results have been reported (15-19).

Little is known about PDGFRA mutations. In general, PDGFRA mutated cases have a low mitotic rate and good prognosis, and most are gastric GISTs (4,20). While CD117 expression is considered a sensitive marker for KIT activation, there is no consensus concerning the reliability of PDGFRA antibody as a marker (21). However, PDGFRA expression has been detected by immunohistochemistry in a number of tumors, and these results have been confirmed by immunoprecipitation/Western blotting experiments (22,23). Notably, a strong PDGFRA immunoreactivity with prominent perinuclear ‘dotlike’ accentuation (so-called Golgi pattern) has been reported to be closely associated with PDGFRA mutations (24,25), and was recently found in 70.3% of PDGFRA mutated cases (26).

The life expectancy of metastatic GIST patients has dramatically changed due to the development of target-based molecular therapy with imatinib mesylate and other tyrosine kinase inhibitors. The determination of clinicopathological and molecular factors predictive of aggressive behavior is therefore of key importance.

In the present study, we analyzed PDGFRA and KIT mutations as well as PDGFRA immunohistochemical expression in 40 patients with GIST in order to investigate the association of the type of mutation and PDGFRA immunostaining with clinicopathologic features and disease prognosis.

**Materials and methods**

Patients and treatment. A total of 40 adult patients with newly diagnosed GISTs, admitted to the Division of Surgical...
Oncology, A.O.U. San Giovanni Battista, Turin, Italy, from 1996 to 2006, were included in the study. There were 19 females and 21 males; the mean age was 64.3 years (range 19-84). Diagnosis was established according to the criteria of the 2004 GIST consensus conference (27). Twenty-nine tumors were located in the stomach, 9 in the small bowel and 2 in the large bowel. The mean size of the tumors was 6.3 cm (range 1-25). According to Fletcher et al (28), the tumors were classified based on the risk of aggressive behavior: 4 were very low risk, 8 low risk, 11 intermediate risk and 17 high risk. GISTS were subtyped into three histopathologic categories: spindle cell, epithelioid or mixed type (28).

After providing their informed consent, the patients underwent surgical resection (5 complete radical, 32 limited complete and 3 incomplete); 8 also received imatinib mesylate. Metastases were present at diagnosis in 3 cases. The follow-up was completed on June 30, 2008. The mean follow-up for the whole series was 39.6 months (range 2-99). Ten (25%) patients relapsed; 5 (12.5%) succumbed to the disease. Only the disease-free survival (DFS) was considered in the analysis.

Immunohistochemistry. Immunohistochemistry was performed on 3-μm-thick dewaxed sections using the Labeled Streptavidin-Biotin 2 System detection kit (Dako, Glostrup, Denmark), diaminobenzidine as chromogen and the monoclonal antibodies anti CD34 (Clone QBEnd/10; Neomarkers, Freemont, CA, USA), dilution 1:50; Smooth Muscle Actin (Clone A4; Dako), dilution 1:50; Vimentin (Clone V9; Dako), dilution 1:100; Desmin (Clone D33; Dako), dilution 1:50; CD44 (clone DF485; Dako), dilution 1:30; Ki-67 Antigen (Clone MIB-1; Dako), dilution 1:100; and polyclonal antibodies anti CD117 (Dako), dilution 1:50; S100 (Dako), dilution 1:4,000, and PDGFRA (Cell Signaling, Danvers, MA, USA), dilution 1:200, following the manufacturer's instructions. In particular, PDGFRA was applied after antigen retrieval using heat-induced epitope retrieval (HIER) in a pressure cooker for 2 min in 5 mM of EDTA buffer, pH 9, after reaching the chamber pressure of 15-25 PSI and temperature of 120°C.

PDGFRA immunostaining was independently evaluated by two pathologists (A.B. and A.F.), who had no knowledge of the tumor clinicopathological data and patient survival. Staining was scored as negative, weakly positive (Fig. 1A), moderately positive (Fig. 1B) and strongly positive (dotlike) (Fig. 1C).

For MIB-1 immunoreactivity, the absolute percentage of stained cells among at least 1,000 cells from the most active areas was recorded, and a cut-off value of 5% was used.

Molecular analysis. Genomic DNA was isolated from formalin-fixed paraffin-embedded tissue using xylene-ethanol for section deparaffinization and standard proteinase K digestion (overnight at 55°C), followed by extraction using the phenol/chloroform method. The quality of the DNA extracted from the tumor tissue was tested by amplification of a 300-bp fragment of the human MHC class II (HLA-DRB) gene using the forward primer 5'-CCG GTC GAC TGT CCC CCC AGC ACG TTT C-3' and reverse primer 5'-GAA TTC TCC CGG CTG CAC TGT GAA GC-3'. PCR amplification of exons 9, 11, 13 and 17 of the KIT gene (15,29), and of exons 12 and 18 of the PDGFRA gene (30,31), was carried out as previously described. PCR products were directly purified using paramagnetic bead technology Ampure (Agencourt Bioscience Corp., Beckman Coulter S.p.A, Milan, Italy) according to the manufacturer's protocol. Direct sequencing of the templates was carried out using the BigDye Terminator Cycle Sequencing Ready Reaction kit v1.1 (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Sequencing reactions were purified using CleanSeq magnetic beads (Agencourt Bioscience Corp., Beckman Coulter S.p.A) according to the manufacturer's protocol, and run on an ABI PRISM 310 automated capillary system (Applied Biosystems).

Statistical analysis. Independence between categorical variables and the type of KIT and PDGFRA mutation were estimated by the Yates' corrected χ² test. Associations between tumor size, mitotic index and Ki67 score, and the type of KIT and PDGFRA mutation were assessed by one-way analysis of variance (ANOVA). Univariate DFS analysis was based on the Kaplan-Meier product-limit estimates of survival distribution (32), and differences between survival curves were tested using the generalized Wilcoxon test. All data were processed with BMDP selected programs (2D, 3D, 7D, 4F and 1L) (33).

Results

Among the 40 GISTS, 26 (65%) carried KIT mutations and 14 (35%) were KIT wild-type. There were 13 deletions, 6 substitutions (point mutations), 3 deletion-substitutions (deletion plus substitution), 3 duplications and 1 insertion. Twenty-five mutations occurred in exon 11 and 1 in exon 9. KIT mutations were grouped into two risk groups. The low-risk (LR) group included 12 cases with substitutions (6), deletion-substitutions (3) and duplications (3). The high-risk (HR) group included 14 cases with deletions (13) and an insertion (1).

PDGFRA mutations were found in 6 out of 40 cases (15%); 34 (85%) were wild-type for PDGFRA. There were 4 deletions and 2 substitutions (point mutations). Five mutations occurred in exon 18 and 1 in exon 12. Due to the small number of mutated cases, PDGFRA mutations were considered as a single group. Eight patients (20%) were KIT and PDGFRA wild-type.

PDGFRA immunopositivity was found in 23 cases (57.5%), 5 of which (12.5%) showed strong immunoreactivity with prominent perinuclear 'dotlike' accentuation (Fig. 1C).

Association between type of KIT/PDGFRA mutation and clinicopathological variables. GISTS with PDGFRA mutations had a smaller size and a lower mitotic index (MI) and Ki67 score than tumors with KIT HR (p=0.01).

At diagnosis, 83.3% of patients with PDGFRA mutations were asymptomatic, in contrast to only 25% of KIT/PDGFRA wild-type, 21.4% of KIT HR and 16.7% of KIT LR patients (p=0.02). Adjacent organ invasion at diagnosis was found in 35.7% of KIT HR and in 12.5% of KIT/PDGFRA wild-type cases, but not in PDGFRA mutated or KIT LR cases (p=0.04). Distant metastasis occurred in 50% of KIT HR and in 37.5% of KIT/PDGFRA wild-type cases, but in only 16.7% of PDGFRA mutated patients; no metastasis was observed in patients with KIT LR (p=0.03). Additionally, relapse occurred in 50% of KIT/PDGFRA wild-type and in 35.7% of KIT HR cases, but
in only 16.7% of PDGFRA mutated cases; no relapse occurred in patients with KIT LR (p=0.05).

PDGFRA immunopositivity was found in all PDGFRA mutated cases, and in 62.5% of KIT/PDGFRA wild-type, 58.3% of KIT LR and only 35.7% of KIT HR cases. The difference between these results is of borderline significance (p=0.06). Notably, PDGFRA ‘dotlike’ immunopositivity (Fig. 1C) was found in 5 out of 6 (83.3%) cases with PDGFRA mutations, but not in KIT HR, KIT LR or KIT/PDGFRA wild-type cases (p=0.0001). All ‘dotlike’ immunopositive cases were wild-type for KIT; 4 carried a PDGFRA deletion and 1 a PDGFRA substitution (point mutation).

The results are shown in Table I.

Discussion

The results of the present study show that GISTs bearing KIT HR mutations were larger and had a higher MI than GISTs with KIT LR mutations. Also, 64.3% of KIT HR patients were at high risk of aggressive tumor behavior, as compared to 33.3% of those with KIT LR (p=0.04); 35.7% presented with invasion into adjacent organs and relapsed, while no patients with KIT LR showed organ invasion or relapsed (p=0.03); distant metastases were found in 50% of KIT HR, but in no KIT LR cases (p=0.003). Furthermore, only 53% of KIT HR patients were free of disease at the 5-year follow-up, in contrast to 100% of the KIT LR cases (p=0.05).

Our findings are in accordance with several studies reporting a poor prognosis for GISTs with KIT deletions (5-13) or insertions (5,11,13), particularly those affecting the 557/558 codon. Indeed, our 2 cases with deletions in codons 557-558 showed metastatic disease at diagnosis and died at 6 and 11 months after surgery, respectively. However, our results are contradictory to a few studies reporting that KIT mutations are a ubiquitous feature of GISTs, either malignant or benign (15,16), and that the type of KIT mutation has no prognostic value (17). Moreover, KIT duplications have been described in a few malignant advanced GISTs in series from imatinib trials (18,19).

PDGFRA mutations were associated with rather indolent tumors; indeed, PDGFRA mutated GISTs had a smaller size and lower MI and Ki67 scores than GISTs with KIT HR mutations. Only 1 patient presented with symptoms (contrary to 80% of patients with KIT mutations; p=0.02); none were at high risk of aggressive tumor behavior or experienced invasion into the adjacent organs; only 1 developed metastasis or relapsed; lastly, all were free of disease at the 40-month follow-up. Our results are in line with a few reports showing a favorable outcome for GISTs with PDGFRA mutations (4,20). However, all cases with PDGFRA mutations were gastric GISTs, and it is known that gastric GISTs have a rather good prognosis. In our series, too, the median survival for the 27 gastric GISTs was 70.8 vs. 13.5 months for the 9 GISTs located in the small bowel and 4.8 months for those located in the large bowel (p=0.0004).

Notably, PDGFRA immunostaining tended to be associated with KIT or PDGFRA mutational status; indeed, positive PDGFRA immunostaining was present in all
PDGFRA mutated cases, but in only 35.7% of KIT HR, 58.3% of KIT LR and 62.5% of KIT/PDGFRα wild-type cases. In particular, a strong perinuclear ‘dotlike’ staining (Golgi pattern) was found in 83.3% of PDGFRA mutated cases, but not in those with KIT mutations or in KIT/PDGFRα wild-type cases. Our findings confirm previous reports (24-26) and indicate that this peculiar PDGFRA-positive staining is a rather specific marker of PDGFRA mutations, and is typically absent in all KIT mutated cases. Further study is required to clarify the biological meaning of the ‘dotlike’ decoration.
The importance of PDGFRA immunostaining in GISTs is further supported by its prognostic value: 7 of 17 immunonegative cases (41.2%) relapsed, while only 3 of 23 (13%) immunopositive cases did (p=0.04). Moreover, at the 5-year follow-up, 72% of PDGFRA immunopositive patients were free of disease, while only 42% of immunonegative patients were.

In conclusion, with the limitation due to the relatively small number of cases, our results confirm that the type of KIT mutation is associated with various biological and clinical behaviors of GISTs, and that PDGFRA mutations are associated with rather benign tumors. They also indicate that a strong PDGFRA immunopositivity reflects PDGFRA mutational status in GISTs and is associated with a good prognosis. Therefore, PDGFRA immunostaining should be a useful additional marker in the diagnostic and prognostic evaluation of GISTs.

Acknowledgements

This study was supported by grants from the Italian Ministero dell’Università e Ricerca Scientifica e Tecnologica (MURST).

Table II. Correlation of type of KIT/PDGFRA mutation and PDGFRA immunostaining with disease-free survival in GISTs.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>1-year DFS rate (%)</th>
<th>3-year DFS rate (%)</th>
<th>5-year DFS rate (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole series</td>
<td>40</td>
<td>92</td>
<td>81</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>PDGFRA deletions/substitutions</td>
<td>6</td>
<td>100</td>
<td>100</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>KIT LR (substitutions/deletion-substitutions/duplications)</td>
<td>12</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>KIT HR (deletions/insertion)</td>
<td>14</td>
<td>85</td>
<td>53</td>
<td>53</td>
<td>0.10</td>
</tr>
<tr>
<td>KIT/PDGFRA wild-type</td>
<td>8</td>
<td>87</td>
<td>87</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>PDGFRA immunostaining</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>17</td>
<td>87</td>
<td>63</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>23</td>
<td>95</td>
<td>95</td>
<td>72</td>
<td>0.04</td>
</tr>
</tbody>
</table>

DFS, disease-free survival; HR, high-risk group; LR, low-risk group. a vs. b, p=0.05; a vs. c, p=0.01

References


