Original article

Gastrointestinal stromal tumors (GISTs): CD117, DOG-1 and PKCθ expression. Is there any advantage in using several markers?

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A B S T R A C T
Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the digestive tract. Expression of CD117, DOG1 and PKCθ was investigated immunohistochemically in a series of 99 paraffin-embedded GISTs in order to determine the sensitivity and diagnostic value of these markers. KIT exons 9, 11, 13 and 17 and PDGFRA exons 12 and 18 were amplified by PCR and sequenced. A total of 94/99 (94%) GISTs stained positive for CD117, 81/99 (82%) for PKCθ and 90/99 (91%) for DOG-1. A significant correlation was noted between CD117 and DOG-1 expression (p = 0.0001). All three markers were expressed in 74% (73/99) of GISTs. Of the five CD117-negative cases, two were PKCθ-negative/DOG1-negative and had mutations in KIT exon 11. Two were PKCθ-positive/DOG1-positive and had mutations in PDGFRA (one each in exons 12 and 18), and one was DOG1-negative/PKCθ-positive, with a PDGFRA exon 18 mutation. The most sensitive marker was CD117, followed by DOG-1 and PKCθ. Although PKCθ was less sensitive, and its staining is more challenging and difficult to interpret, the use of this marker is highly recommended, particularly in CD117-negative/DOG-1-negative GISTs.

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Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the digestive tract and show lineage differentiation along the lines of the interstitial cells of Cajal [1]. They are generally KIT (CD117)-positive, and display KIT or PDGFRA gene mutations [2,3]. Mutations in other genes, such as BRAF [4] and SDHB [5], have been reported more rarely. These tumors have recently prompted particular interest due to their good response to new targeted therapies [6,7].

Although CD117 positivity has traditionally been seen as the gold standard for GIST diagnosis and, at least in early clinical trials, as an eligibility criterion for imatinib therapy [8], about 4–10% of GISTs express little or no CD117 [9–11]. Research has focused on the mutational status of these CD117-negative cases [9,12,13], and has sought to establish the value of other markers discovered in molecular studies [14–20]. Today, it is widely accepted that CD117-negative GISTs are a heterogenous group of neoplasms that frequently display KIT or PDGFRA mutations and may also express DOG1 and/or PKCθ. However, few comparative reports have been published [20].

This study investigated CD117, DOG1 and PKCθ expression in a series of 99 GISTs in order to determine the sensitivity and diagnostic value of these markers.

Materials and methods

Patient selection and clinical features

Representative samples were selected from a set of formalin-fixed, paraffin-embedded surgical specimens obtained from 99 GISTs. Samples were from patients undergoing surgery at the Hospital Universitario Virgen Macarena (Seville) and Hospital Torrecárdenas (Almería) over a 20-year period (1989–2009). None of the patients had previously received treatment with imatinib. Tumor sample collection was approved by the Hospital Ethical Committees. Hematoxylin–eosin-stained slides were reviewed, and the pathological diagnosis was confirmed by tumor location, morphology, immunostaining for CD117 and molecular analysis of KIT/PDGFRA status. Tissue microarrays (TMAs) were constructed using a TMADeveloper® version 1.1 manual tissue arrayer (Alphelys, Plaisir, France); five 0.6 mm cores were taken from each paraffin-embedded sample.
Table 1
Antibodies used for immunohistochemical analysis.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD117</td>
<td>Polyclonal (Diagnostic Biosoystm, CA)</td>
<td>Prediluted</td>
</tr>
<tr>
<td>DOG-1</td>
<td>Monoclonal (Clone K9, Novocastra, UK)</td>
<td>Prediluted</td>
</tr>
<tr>
<td>PKCα</td>
<td>Monoclonal (clone 27, BD Bioscience, USA)</td>
<td>1:100</td>
</tr>
</tbody>
</table>

The following clinical and pathological data were accessed: patient age and gender, tumor location (gastric, small and large bowel, extragastrointestinal), tumor size, histological type (spindle, epithelioid, mixed), and mitotic index in 50HPF.

Immunohistochemical analysis

For immunohistochemical analysis, 5 μm serial sections were stained with a panel of antibodies (Table 1) using the streptavidin–biotin-peroxidase complex technique. Negative (primary antibody replaced by normal horse serum) and positive controls were included in each slide run. All controls yielded satisfactory results. Specimens with diffuse or focal immunostaining were considered positive. Tumors with ≤5% of positive cells were considered negative. Staining for individual antibodies was classified as either positive or negative.

DNA isolation and molecular analysis

One selected paraffin block was chosen for molecular analysis. DNA was isolated using a QiAmp DNA FFPE Tissue kit according to the manufacturer’s instructions (Qiagen, Hilden, Germany). KIT exons 9, 11, 13 and 17 and PDGFRα exons 12 and 18 were amplified by polymerase chain reaction (PCR) using a Taq PCR Master Mix (Qiagen, Hilden, Germany), including 0.1–1 μg of extracted DNA and 0.2 μM forward and reverse primers to a total volume of 30 μL. Primers and experimental conditions are shown in Table 2. PCR products were examined using a QuiaXcel DNA High Resolution kit (Qiagen, Hilden, Germany), and sequencing was carried out with an automated analyzer (ABI PRISM 3130×l Genetic Analyzer, Applied Biosystem, USA). KIT and PDGFRα sequencing results were compared with gene sequences in the NCBI genebank.

Statistical analysis

Clinical and immunohistochemical data were analyzed using the SPSS for Windows, version 17.0 software package (SPSS Inc., Chicago, IL, USA). Chi-square and Fisher tests were used to analyze associations between variables. A p-value of less than 0.05 was considered statistically significant.

Table 2
Primer used for PCR. Primers are presented in 5′ to 3′ orientation with coding strand labeled (+) and noncoding strand labeled (−). \( T_m \) and cycle number corresponding to the linear part of the amplification.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Strand</th>
<th>( T_m ) (°C)</th>
<th>Cycle no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIT exon 9</td>
<td>TCTAGAGCTAGTAAAGCAGGGGCTT</td>
<td>−</td>
<td>56 C, 40x</td>
</tr>
<tr>
<td></td>
<td>TGGTAGACAGACGCTAAACATCC</td>
<td>+</td>
<td>56°C, 40x</td>
</tr>
<tr>
<td>KIT exon 11</td>
<td>CCAGCTGCTCTATAGACTG</td>
<td>−</td>
<td>56°C, 40x</td>
</tr>
<tr>
<td></td>
<td>AGCCCTGGTTCTACAGGAS</td>
<td>+</td>
<td>56°C, 40x</td>
</tr>
<tr>
<td>KIT exon 13</td>
<td>GACATCAGTTTCTACAGT</td>
<td>−</td>
<td>56°C, 40x</td>
</tr>
<tr>
<td></td>
<td>GCAAGAAGACAAACAG</td>
<td>+</td>
<td>56°C, 40x</td>
</tr>
<tr>
<td>KIT exon 17</td>
<td>GTTAAATTATAGCAAGCGG</td>
<td>−</td>
<td>56°C, 40x</td>
</tr>
<tr>
<td></td>
<td>TTAGATTAGAAATGCAAGG</td>
<td>+</td>
<td>56°C, 40x</td>
</tr>
<tr>
<td>PDGFRα exon 12</td>
<td>TCAAGTCTGCATCTCGTCT</td>
<td>−</td>
<td>56°C, 40x</td>
</tr>
<tr>
<td></td>
<td>GCAAGGAGAAAGGAGCTCTT</td>
<td>+</td>
<td>56°C, 40x</td>
</tr>
<tr>
<td>PDGFRα exon 18</td>
<td>ACCATGATCGAGGATCGTCTT</td>
<td>+</td>
<td>56°C, 40x</td>
</tr>
<tr>
<td></td>
<td>TGAAGGAGATGACCGTACC</td>
<td>+</td>
<td>56°C, 40x</td>
</tr>
</tbody>
</table>

Results

Clinical features and mutational status

All tumors displayed clinical/pathological features consistent with GIST; all expressed CD117 and/or harbored KIT/PDGFRα mutations. The median age was 64 years (range: 13–87), and samples were from 51 (51.5%) males and 48 (48.5%) females. Only two patients were under 20 years old. Forty-eight (48.5%) tumors originated in the stomach, 36 (36.4%) in the small bowel, 7 (7.1%) in the large bowel and 8 (8.2%) in extragastrointestinal locations in the mesentery and omentum. Mean tumor diameter was 7.65 cm (1.0–25 cm). Tumor diameter was less than 2 cm in 61 (61.6%) cases, between 2 and 5 cm in 30 cases (30.3%), between 5 and 10 cm in 6 cases (6.1%) and over 10 cm in 2 cases (2.0%). Histologically, 79 (80%) neoplasms were of spindle type, 15 (15%) of epithelioid type, and 5 (5%) showed mixed cytomorphology. Mitotic counts ranged from 1 to 60 in 50 high power fields (50HPF): 54 samples (55%) displayed <2 mitotic counts; 17 (17%) between 2 and 5; and 28 (28%) >5.

Molecular analysis revealed KIT mutation in 68/99 (69%) cases, PDGFRα mutation in 11/99 (11%) and wild type (wt) KIT/PDGFRα in 20/99 (20%). Among cases with KIT mutation, 64/68 (94%) had exon 11 mutations and 4/68 (6%) exon 9 mutations. There were no cases of exon 13 or exon 17 mutation. Among cases with PDGFRα mutation, 9/11 (82%) had mutations of exon 18 and 2/11 (18%) of exon 12. All CD117-negative cases had KIT (2, exon 11) or PDGFRα (2, exon 18; 1, exon 12) mutations.

Immunohistochemical analysis of CD117, DOG-1 and PKCα expression

CD117+ staining was observed in 94/99 (94%) cases with the following distribution: stomach, 43/47 (91.5%), small bowel, 34/35 (97.1%), large bowel 8/8 (100%), extragastrointestinal 9/9 (100%). In most cases, the staining was diffuse, uniform and strong. Occasionally, however, focal and/or weak staining was detected (Fig. 1). The staining pattern was cytoplasmic, sometimes with membrane reinforcement; however, a remarkable Golgi-like dotting pattern was also observed in 44/99 (44.5%) cases (Fig. 2).

PKCα+ staining was detected in 81/99 (82%) cases, with the following distribution: stomach 35/47 (75%), small bowel 32/34 (94%), large bowel 7/8 (87.5%) and extragastrointestinal 7/9 (77.8%). The staining pattern was cytoplasmic and mostly diffuse, and intensity was generally weak to moderate (Fig. 3). In some cases, however, a focal staining pattern was observed (Fig. 4). PKC0-negative cases were located mainly in the stomach (12/18), and almost all of them (11/12) were CD117-positive.

Three of the five CD117-negative GISTs (60%) were PKC0-positive. All three were located in the stomach. Of the 94 CD117-positive GISTs, a total of 78 (83%) also expressed PKC0. Most
PKC and CD117 expression and mutational status.

<table>
<thead>
<tr>
<th>PKC0/CD117+</th>
<th>KIT exon 9</th>
<th>KIT exon 11</th>
<th>PDGFRA exon 18</th>
<th>PDGFRA exon 12</th>
<th>Wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKC0+/CD117+</td>
<td>4/78 (5%)</td>
<td>53/78 (68%)</td>
<td>1/78 (1%)</td>
<td>5/78 (7%)</td>
<td>15/78 (19%)</td>
</tr>
<tr>
<td>PKC0+/CD117−</td>
<td>0/3 (0%)</td>
<td>0/3 (0%)</td>
<td>1/3 (34%)</td>
<td>2/3 (67%)</td>
<td>0/3 (0%)</td>
</tr>
<tr>
<td>PKC−/CD117+</td>
<td>0/16 (0%)</td>
<td>9/16 (56.25%)</td>
<td>0/16 (0%)</td>
<td>2/16 (12.5%)</td>
<td>5/16 (31.25%)</td>
</tr>
<tr>
<td>PKC−/CD117−</td>
<td>0/2 (0%)</td>
<td>2/2 (100%)</td>
<td>0/2 (0%)</td>
<td>0/2 (0%)</td>
<td>0/2 (0%)</td>
</tr>
</tbody>
</table>

Staining was predominantly cytoplasmic and strong in spindle-cell lesions, while membrane reinforcement was especially prominent in epithelioid cell lesions (Fig. 5). The staining pattern was generally diffuse, although focal staining was occasionally observed (Fig. 6). No statistically significant correlation was noted between positive staining and anatomical location (p = 0.862).

Correlations between DOG1 and CD117 positivity are shown in Table 5. DOG1 expression was less common than CD117 expression, though not significantly so (90/99 vs. 94/99). A total of 93.5% of CD117-positive GISTs were also DOG1-positive; only two of the five CD117-negative tumors were DOG1-positive (40%). A significant correlation was observed between these markers (p = 0.0001). The two CD117-negative/DOG1-positive cases were located in the stomach and had PDGFRA mutations (exon 12 and exon 18).

Table 3
PKC0 and CD117 expression.

<table>
<thead>
<tr>
<th>PKC0</th>
<th>CD117+</th>
<th>CD117−</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKC0+</td>
<td>78/94 (83%)</td>
<td>3/5 (60%)</td>
<td>81 (83%)</td>
</tr>
<tr>
<td>PKC0−</td>
<td>16/94 (17%)</td>
<td>2/5 (40%)</td>
<td>18 (18%)</td>
</tr>
<tr>
<td>Total</td>
<td>94 (100%)</td>
<td>5 (100%)</td>
<td>99 (100%)</td>
</tr>
</tbody>
</table>

Table 5
DOG-1 and CD117 expression.

<table>
<thead>
<tr>
<th>CD117</th>
<th>DOG-1+</th>
<th>DOG-1−</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD117+</td>
<td>88/89 (93.5%)</td>
<td>6/93 (6.6%)</td>
<td>94 (100%)</td>
</tr>
<tr>
<td>CD117−</td>
<td>2/5 (40%)</td>
<td>3/5 (60%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>90 (90.9%)</td>
<td>9 (9.2%)</td>
<td>99 (100%)</td>
</tr>
</tbody>
</table>

Fig. 1. CD117 staining. (A and B) Serosal GIST, diffuse staining. (C and D) Intramural gastric tumor, strong focal staining.
Six of the nine DOG1-negative GISTs (66.7%) were CD117-positive and harbored KIT mutations in exon 11 (2/6), PDGFRA mutations in exon 12 (1/6), or wild type KIT/PDGFRα (3/6). All except one of the DOG1-negative/CD117-negative GISTs (4/10) had KIT exon 11 mutations (Table 6).

Most GISTs (73/99, 74%) expressed all three markers. Of the five CD117-negative tumors, 2 expressed none of the three markers and had KIT exon 11 mutations. Two were positive for PKC-0 and DOG1, and had PDGFRA mutations at exon 12 and exon 18 (Table 7), while the fifth stained positive only for PKC-0 (PDGFRA exon 18 mutation).

No correlation was found between positivity for CD117, PKC0 or DOG-1 and anatomical location (p = 0.532; p = 0.631; p = 0.862, respectively).

Discussion

This study focused on the expression of three immunohistochemical markers used in the diagnosis of GISTs, in a series of 99 specimens handled routinely at two general hospitals. The percentage of CD117-negative/KIT/PDGFRα-mutated cases (5%) was within the range generally reported in the literature [9,12,13]. CD117 expression was long considered a prerequisite for GIST diagnosis [8], but it is now thought that as many as 4–10% of GISTs express little or no CD117 [9,12,13]. This realization has prompted the use of new markers (chiefly PKC0 and DOG1), which have so far yielded overlapping and sometimes complementary results [18–22].

PKC0, a protein kinase C subtype, plays a major role in T lymphocyte activation, in skeletal muscle signal transduction and in neuronal differentiation [23–26,14]. In the normal digestive tract, positive staining is observed in interstitial cells of Cajal, in mast cells [22,26,14] and in endothelial cells [22]. In GISTs, immunostaining tends to be cytoplasmic and diffusely granular, with occasional Golgi-like perinuclear dotting [14]. Staining intensity is mild to moderate. Reported rates of PKC0 expression vary from 72% to 100%, depending on the specific features of the study [15,16,22,14], and although CD117 and PKC0 expression tend to coincide, GISTs sometimes express only one marker or the other. In CD117-negative GISTs, PKC0 expression is also reported to range from 72% to 100% [15–17,22]. Here, PKC0+ staining was detected overall in 81 of 99 cases (82%). Staining was positive in three out of five CD117-negative GISTs (60%) and in 78 of 94 CD117-positive tumors (83%). It was initially believed that PKC0+ staining was to be found solely in GISTs [14], but it has subsequently been reported in 21.9% of non-GIST tumors [17], including schwannomas (14–57%) [17], leiomyomas [17], leiomyosarcomas (10–28%)
Fig. 3. PKCε expression in the normal gastrointestinal tract (small intestine). (A) Panoramic view showing positivity only in a few myenteric plexus cells. (B) Mucosa (detail): no positive staining in epithelial cells. (C) Myenteric plexus (detail): occasional positive staining in cytoplasm and projections.

[15,17], desmoid tumors (15%) [17], malignant peripheral nerve sheath tumors and Ewing’s sarcoma/primitive neuroectodermal tumors [27].

Some authors do not recommend PKCε as a marker for routine use, due to excessive background staining with current commercial antibodies, limited reproducibility and the difficulty of interpreting staining patterns [18,21]. Major differences have also been reported as a function of antibody type: monoclonal antibodies appear to be fairly specific, but are less sensitive than polyclonal antibodies [15]. Kang et al. note that the best results using monoclonal antibodies are obtained with a 1/200 dilution and antigen retrieval [20]. Here, using a 1/100 dilution and antigen retrieval, results were slightly inferior to those reported by Kang et al. [20] in that staining was uneven and of mild-to-moderate intensity. Even so, the results obtained here suggest that PKCε might be useful in CD117-negative/DOG1-negative GISTs: it was the only marker expressed by one tumor. Similarly overlapping results have also been observed by Kang et al. [20]. It has recently been suggested that PKCε expression may be of therapeutic interest in imatinib-resistant patients treated with sunitinib (Sutent, Pfizer, USA), since this protein is expressed and phosphorylated regardless of KIT or PDGFRα mutational status, and PKCε knockout is associated with inhibition of the PI3K/AKT signaling pathway, upregulation of the kinase inhibitors p21 and p27, and anti-proliferative effects [28].

Table 7

<table>
<thead>
<tr>
<th>CD117-</th>
<th>PKCε-</th>
<th>PKCε+</th>
<th>Total</th>
<th>CD117+</th>
<th>PKCε-</th>
<th>PKCε+</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOG-1-</td>
<td>2 (66.7%)</td>
<td>1 (33.3%)</td>
<td>3</td>
<td>1 (16.7%)</td>
<td>5 (83.3%)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>DOG-1+</td>
<td>0 (0%)</td>
<td>2 (100%)</td>
<td>2</td>
<td>15 (17%)</td>
<td>73 (83%)</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>16</td>
<td>78</td>
<td>94</td>
<td></td>
</tr>
</tbody>
</table>
Moreover, certain aspects of KIT and PKC in GIST cells appear to be interdependent, since PKC binds to KIT and might phosphorylate it, while KIT probably regulates PKC phosphorylation [15].

The gene DOG1 (an acronym for “Discovered on Gist 1”) encodes the transmembrane protein anoctamin (FLJ10261), associated with calcium-dependent chloride channel activity [29–31]. Although its relationship with the KIT and PDGFRA genes remains unclear, DOG1+ staining in interstitial cells of Cajal suggests a possible involvement in the activation of KIT and PDGFRA receptors [28]. In GISTs, both cytoplasmic and membrane staining are reported; though staining is generally strong and diffuse, in 25% of cases, it is noticeably weak, while in 5% of tumors, focal staining is observed in <30% of cells [18]. In the normal digestive tract, positive staining is found only in the interstitial cells of Cajal [18,19] and in gastric...
surface epithelia [18]. West et al. [29] have reported DOG1-positive staining in mast cells using polyclonal antibodies, but these findings have not been confirmed with monoclonal antibodies [18].

In tissue arrays, Espinosa et al. [19] reported DOG1-positive staining in 87% of GISTs, whereas CD117 positivity was detected in only 74%. The difference was even more marked in GISTs with PDGFRα mutations. However, no correlation was found between DOG1 expression, anatomical location or specific mutations. Liegl et al. [21], in a study of DOG1 expression in whole sections from conventional GISTs and unusual subgroups (CD117-negative GISTs, pediatric GISTs and GISTs associated with neurofibromatosis 1), found that DOG1 expression was detected in 36% of CD117-negative GISTs. Using commercial monoclonal antibodies (Clone K9, Novocastra), Miettinen et al. [18] reported that DOG1 positivity rates were virtually identical to CD117 positivity rates (94.8% vs. 94.9%). A total of 92.3% of tumors were positive for both markers, while 2.6% were negative for both. Roughly half the CD-117 negative tumors were positive for DOG1, and vice versa. Variations were also noted as a function of histological type and anatomical location.

The findings of the present study with regard to CD117/DOG1-mutational status combinations were comparable to those reported by Miettinen et al. [18] except in the DOG1-negative/CD117-negative group, where most GISTs were of the wt KIT/PDGFRα genotype.

DOG1 is a highly sensitive marker for GIST diagnosis and displays greater specificity than CD117 [19], but it is by no means specific for this tumor lineage. Sporadic positivity has been reported in desmoplastic melanomas, synovial sarcomas, leiomyosarcomas, basal-cell carcinomas, hepatocellular carcinomas, adenoid cystic carcinomas, bowel adenomas, esophageal squamous cell carcinomas, gastric carcinomas and uterine-type retroperitoneal leiomyomas [18,19]. However, given that between 36% and 50% of CD117-negative tumors are DOG1-positive [18], this antibody should be included in routine histochemical diagnosis of GISTs.

Kang et al. [20] recently studied DOG1 and PKCθ expression in whole sections from 26 CD117-negative GISTs, reporting that 80% of cases expressed both markers; PKCθ was expressed in two DOG1-negative tumors, while DOG1 was expressed in one PKCθ-negative tumor. Although the number of CD117-negative tumors in the present study was considerably lower, and the results obtained are therefore of no statistical significance, the findings highlight the existence of GISTs expressing only PKCθ. The authors therefore believe that staining for PKCθ should be included in the immunohistochemical diagnosis of GISTs, especially in CD117-negative/DOG1-negative tumors.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgment

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References


