A KIT-negative, DOG1-positive Epithelioid GIST of the Stomach Harboring a Novel PDGFRA Exon 14 Single Nucleotide Deletion

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Abstract. Gastrointestinal stromal tumors (GISTs) are the most common primary mesenchymal tumors of the gastrointestinal tract, and most of them harbor KIT or platelet-derived growth factor receptor alpha (PDGFRA) gain-of-function mutations. Proper diagnostic assessment of GISTs has become very important since the availability of the molecular-targeted therapy with imatinib mesylate. Histopathology remains the gold standard in GIST diagnosis, and immunohistochemistry plays the major confirmatory role. Moreover, genetic sequencing not only further confirms the diagnosis of GIST, but also provides information for the optimal treatment of patients. Herein, we describe a gastric GIST harboring a novel PDGFRA exon 14 frameshift mutation caused by a single-nucleotide deletion. The case reported here represents further evidence regarding the existence of a distinct subset of GISTs characterized by the PDGFRA mutation, the gastric localisation, the epithelioid morphology, and the weak or negative immunohistochemical expression of KIT. DOG1 is emerging as a promising biomarker for this subgroup of GISTs.

Once a poorly defined clinicopathological entity, in recent years gastrointestinal stromal tumor (GIST) has emerged as the paradigm of a tumor treatable with molecularly targeted therapy. Better understanding of the molecular and genetic characteristics of GIST has increased the accuracy of its diagnosis and allowed for the identification of distinct genetic hallmarks, prognostic grouping and treatment strategies. Most GISTs harbor activating mutations of the KIT tyrosine kinase, which occur with decreasing frequency in exons 11, 9, 13, and 17. A subset of tumors, especially those with gastric localisation and epithelioid morphology, have mutations in the KIT-related kinase gene platelet-derived growth factor receptor alpha (PDGFRA), which occur most commonly in exon 18. PDGFRA exon 12 and exon 14 are rarely involved. The remainder of GISTs are of wild type for both KIT and PDGFRA (1).

Proper identification of GISTs has become very important since the availability of a novel and effective specific, pathogenesis-targeted treatment with the KIT/PDGFRA tyrosine kinase inhibitor imatinib mesylate. The great majority of GISTs exhibit strong and diffuse positivity for KIT (CD117) and until recently, KIT immunostaining has been the main tool for the diagnosis of GISTs. Nevertheless, a small minority of GISTs, especially those with mutant PDGFRA, exhibit weak or negative expressions of KIT and are, thus, problematic in their diagnosis (2). Anoctamin-1 (ANO1), a chloride-channel protein detected by the DOG1 antibody, is emerging as an equally sensitive and specific marker that can identify one third to one half of the KIT-negative GISTs, with a better sensitivity in detecting epithelioid GISTs and tumors with PDGFRA mutations (3). Furthermore, genetic sequencing is becoming an increasingly important component in GISTs diagnostic assessment because it identifies the tumor as a GIST, confirming the histopathological diagnosis, and provides valuable information for the optimal treatment for patients, since the responsiveness to treatment with imatinib varies substantially depending on the mutation status of the tumor (3).

Herein, we report a case of a gastric polypoid neoplasm with epithelioid morphology. Immunohistochemical staining showed that the tumor was negative for KIT, but strongly and diffusely positive for DOG1. The molecular analysis demonstrated that the lesion harbored a novel PDGFRA gene mutation in exon 14 (p.K666SfsX29), thus confirming the diagnosis of GIST.
Case Report

The patient was a 88-year-old man who was presented to our clinic with upper digestive hemorrhage. His past medical history was uneventful and the physical examination was negative. Upper gastrointestinal endoscopy demonstrated a pedunculated polypoid mass measuring 2 cm in the greatest diameter, located between the distal portion of the gastric body and the antrum. The lesion was removed by endoscopic diathermic knife and was submitted for pathologic evaluation.

Immunohistochemistry. Immunohistochemistry was performed on 4 μm-thick sections of formalin-fixed, paraffin-embedded tumor tissue using the following antibodies: cytokeratin AE1/AE3 (1:200, pH 6), S-100 protein (1:1000), CD45 (1:50, pH 6), synaptophysin (1:200, pH 6), neurofilaments (1:200, pH 6), muscle-specific actin HHF 35 (1:200, pH 6), myogenin (1:50, pH 6), smooth muscle actin 1a4 (1:200, pH 6), CD34 (1:100, pH 6), CD117 (1:100), MIB-1 (1:50, pH 6); all from Dako, Carpinteria, CA, USA), DOG1 (1:100, pH 6; Aczon, Bologna, Italy), alpha-sarcomeric actin (1:1000, pH 6; Sigma-Aldrich, Steinheim, Germany). Immunohistochemical staining was automated using the Autostainer Link 48 by DakoCytomation (Dako). For antigen retrieval, EnVision™ FLEX Target Retrieval Solution of low pH (Dako) was automated using the Autostainer Link 48 by DakoCytomation (Dako). For antigen retrieval, EnVision™ FLEX Target Retrieval Solution of low pH (Dako) was employed for 30 min at 98°C. No antigen retrieval techniques were used for the S-100 and the CD117 protein staining.

Molecular analysis. DNA was extracted from formalin-fixed, paraffin-embedded tumor tissues. Five 10 μm-thick sections were deparaffinized by serial xylene/ethanol washing and DNA extraction was completed using the QIAamp DNA kit (QIAGen GmbH, Hilden, Germany). Exons 9, 11, 13 and 17 of the PDGFRA gene were sequenced using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). PCR conditions were as follows: an initial denaturation step of 95°C for 5 min followed by 40 cycles at 95°C/30 s, 60°C/30 s and 72°C/30 s.

The PCR conditions for exon 18 of PDGFRA were 40 cycles at 95°C/15 s, 60°C/15 s and 72°C/15 s. Exon 14 of the PDGFRA gene was amplified in duplicate and sequencing in forward and reverse was performed using the independent PCR product. DNA from normal tissues (prostate) was analyzed in order to exclude a rare polymorphism.

Results

Gross and microscopic morphology. Grossly, the material submitted for pathological evaluation consisted of two polypoid fragments measuring respectively 2.6 cm and 1.9 cm in the greatest diameter. Microscopically, the fragments were composed of gastric wall, which was extensively ulcerated due to the presence of a well-circumscribed but non-encapsulated, expansile nodular lesion in the muscularis propria, measuring 1.5 cm in the greatest diameter. The tumor was moderately cellular and composed of discohesive epithelioid cells embedded in an abundant myxoid matrix, with numerous thin-walled vessels present throughout, had areas of hemorrhage and hemosiderin deposits. Tumor-infiltrating collection of lymphocytes were focally present. The tumor cells were polymorphous, for the most part round or oval with eosinophilic cytoplasm, they had distinct cellular borders and peripherally placed nuclei. Giant multinucleated cells and rhabdoid cells were frequently seen. There was no nuclear atypia and no necrosis, and mitotic figures were sparse. The histology of the tumor is shown in Figure 1.

Immunohistochemical staining. Immunohistochemistry revealed that the tumor cells were negative for broad-spectrum cytokeratins AE1/AE3, S-100 protein, CD45, synaptophysin, neurofilaments, muscle-specific actin HHF 35, myogenin, CD34, and CD117. Staining for alpha-sarcomeric actin was diffusely positive, with more intense staining in the rhabdoid cells. The staining for smooth muscle actin was focally positive. DOG1 was diffusely and strongly expressed, with a membranous and Golgi (perinuclear dot-like) pattern, as shown in Figure 2. The Ki-67 (MIB-1) labeling index was 10-15%.

Molecular analysis. Molecular analysis demonstrated a PDGFRA exon 14 frameshift mutation caused by a single-nucleotide deletion (c.2135delA) resulting in a premature stop codon (p.K666SfsX29). KIT exons 9, 11, 13 and 17 and PDGFRA exon 12 and 18 were of wild type.

Diagnostic conclusion. The final diagnosis was GIST of epithelioid type, pT1 NX. The mitotic count revealed 3 mitotic figures per 50 hpf. The small size of the lesion and its low mitotic rate indicated very low risk for aggressive behavior (4).

Discussion

GISTs are the most common primary mesenchymal tumors of the gastrointestinal tract and are thought to originate from interstitial cells of Cajal or their stem cell precursors. Approximately 60% of GISTs arise in the stomach and 25% of these are malignant. Gastric GISTs have a broad histological spectrum. Most are spindle cell tumors, while epithelioid morphology is seen in 20-25% of cases, with a number of cases having a mixed morphology (1). In particular, PDGFRA-mutated GISTs were shown to have several morphological features distinguishing them from KIT-mutated tumors, namely, epithelioid or mixed pattern, low to moderate cellularity, prominent myxoid change, increased cellular pleomorphism, low mitotic count, tumor-infiltrating mast cells, and tumor-infiltrating lymphocytes (5).
Accurate diagnosis of GIST patients has become crucial since the relatively recent development of the tyrosine kinase inhibitor imatinib mesylate, which has revolutionized the treatment of malignant GISTs. Histopathology remains the gold standard in GIST diagnosis, with immunohistochemistry playing a major confirmatory diagnostic role. Most gastric GISTs exhibit strong positivity for KIT (CD117), which appears to be cytoplasmic, membrane-associated, or sometimes in the form of perinuclear dots. KIT immunostaining has been a major step forward in the reliable and reproducible diagnosis of GIST, but a small minority of cases (<5%), especially GISTs with mutant PDGFRA, may have very limited, if any, positivity (2). The calcium-activated chloride channel protein anoctamin-1 (ANO1), detected by the DOG1 antibody, has emerged in recent years as a promising biomarker for GISTs. The results of several studies have shown that DOG1 is more sensitive and specific than KIT in the detection of GISTs and that it can identify one third to one half of the KIT-negative GISTs, especially tumors of gastric origin, tumors with epithelioid morphology, and tumors harboring the PDGFRA mutation. These findings support the use of DOG1 in combination with KIT as diagnostic markers for GIST (3). Moreover, most spindle cell GISTs are positive for CD34, whereas epithelioid examples are less frequently positive. A minority of gastric GISTs express smooth muscle actin and rare examples exhibit positivity for desmin, keratins (usually limited to keratin 18), or the S-100 protein (1).

Despite clinicopathological differences, most GISTs share a similar genetic profile, including gain-of-function mutations of KIT or PDGFRA. Results from KIT and PDGFRA gene mutation analysis can be used not only to support the histological diagnosis of GIST, but also to predict the likelihood...
of tumor resistance to tyrosine kinase receptor inhibitors and to fine-tune the dosage of imatinib used to treat an individual tumor. KIT mutations are usually detected in exon 11 and, in order of decreasing frequency, they include in-frame deletions, single-nucleotide substitutions, duplications, and insertions. KIT exons 9, 13, and 17 are rarely involved. Most KIT-mutant GISTs are sensitive to imatinib. However, exon 17 KIT-mutants are primarily resistant, and exon 9 KIT-mutants are less sensitive than exon 11 mutants. A subset of GISTs, especially tumors with gastric location and epithelioid morphology, have mutations in PDGFRA, which is closely homologous to KIT. These mutations include single-nucleotide substitutions and in-frame deletions and involve PDGFRA exon 18 or exon 12. The majority occur in exon 18 and lead to substitution D842V, which is characterized by notable resistance of the tumor to imatinib. Mutations in PDGFRA exon 14 are very rare and include single-nucleotide substitutions clustered in codon 659 (1, 6). They seem to be associated with low KIT expression and an unexpectedly favorable prognosis (7). More recently, a single nucleotide substitution, S667P has been described (8). Furthermore, a point mutation in one of the well-known hot-spots of the BRAF gene has also been described in a small subset of GISTs (9).

In the reported case, the tumor harbored a PDGFRA exon 14 frameshift mutation caused by a single-nucleotide deletion (c.2135delA) resulting in a premature stop codon (p.K666SfsX29). To the best of our knowledge, this mutation has never been described for PDGFRA (9).

The tumor described in this report is a paradigmatic example of a PDGFRA-mutated GIST, characterized by gastric location, epithelioid morphology, negative immunohistochemical expression of KIT (CD117), and wild type KIT at the molecular level. It represents further evidence that GISTs harboring PDGFRA mutations are a distinct subset of tumors with well-defined clinicopathological features, which raises new challenges with regards to diagnosis and treatment with kinase inhibitors (2, 7, 10-15). A possible emerging role of DOG1 in the identification of GISTs belonging to this subgroup is also emphasized.

References