

Case Report

Myxoid epithelioid gastrointestinal stromal tumor harboring an unreported *PDGFRA* mutation: report of a case and review of the literature

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Abstract: Activating mutations of platelet-derived growth factor receptor α (*PDGFRA*) are detected in a significant proportion of gastrointestinal stromal tumors (GISTs), in addition to the more frequent mutation in *c-kit*. GISTs with *PDGFRA* mutations have been found to have several characteristic morphological features, sometimes allowing to discriminate them from GISTs with *c-kit* mutations. Among these, epithelioid morphology in tumor cells and tumor-infiltrating mast cells are powerful predictors of *PDGFRA* mutations. Although myxoid stroma by itself is not so much a reliable predictor of *PDGFRA* mutation, myxoid stroma in conjunction with epithelioid morphology in tumor cells is a powerful predictor of mutations in this gene. GISTs showing either weak or negative immunoreactivity for *c-kit* and epithelioid cells with myxoid stroma are called myxoid epithelioid GISTs, which typically show *PDGFRA* mutation. Herein, we presented a case of a 59-year-old woman with myxoid epithelioid GIST of the stomach. A unique finding in this case was eosinophil infiltration, probably more numerous than mast cells; mast cell infiltration is known to be usually found in myxoid epithelioid GIST. The existence of a similar mechanism in eosinophil and mast cell recruitment via tumor-producing stem cell factor is speculated. Mutational analyses revealed a *PDGFRA* exon 18 mutation: D842_H845del, D846N. Combined deletion and substitution mutation has been reported in rare instances, but to the best of our knowledge, D846N has not been documented.

Keywords: Myxoid epithelioid gastrointestinal stromal tumor, mutation, *PDGFRA* gene, exon 18

Introduction

In 1998, expression of *c-kit* protein in conjunction with *c-kit* “gain of function” mutations was revealed to be a diagnostic hallmark of gastrointestinal stromal tumor (GIST) and to be involved in tumorigenesis of GIST [1, 2]. A subset of GISTs, however, did not express *c-kit* and/or *c-kit* wild type.

In 2003, activating mutations of platelet-derived growth factor receptor α (*PDGFRA*) were detected in a significant proportion of these *c-kit* wild type GISTs [3, 4]. GISTs with *PDGFRA* mutations were found to have several characteristic morphological features, sometimes allowing to discriminate them from GISTs with *c-kit* mutations, such as epithelioid pattern [5-8], myxoid stroma [9], tumor-infiltrating mast cells [9], multinucleated neoplastic cells [10], and rhabdoid cells [11].

The definition of myxoid epithelioid GISTs was proposed in 2005 by Sakurai et al. [9]. In their study, different histopathological patterns were observed within the epithelioid tumor cells in GISTs with no or low expression of *c-kit*. Some of them showed tightly cohesive growth of epithelioid tumor cells. The others exhibited loose arrangement of epithelioid tumor cells within a background of myxoid stroma. GISTs of this latter type were said to belong to the myxoid epithelioid group and to be closely associated with mast cell infiltration and *PDGFRA* mutations. Myxoid epithelioid GISTs are largely located in the stomach, with a minority in the omentum [9]. In general, *PDGFRA*-mutated GISTs are likely to occur in the stomach [12].

With regards to *PDGFRA* exon 18 mutations in GISTs, D842V or D842_H845del were the two mutations most frequently encountered, including in myxoid epithelioid GISTs [9, 11, 13].

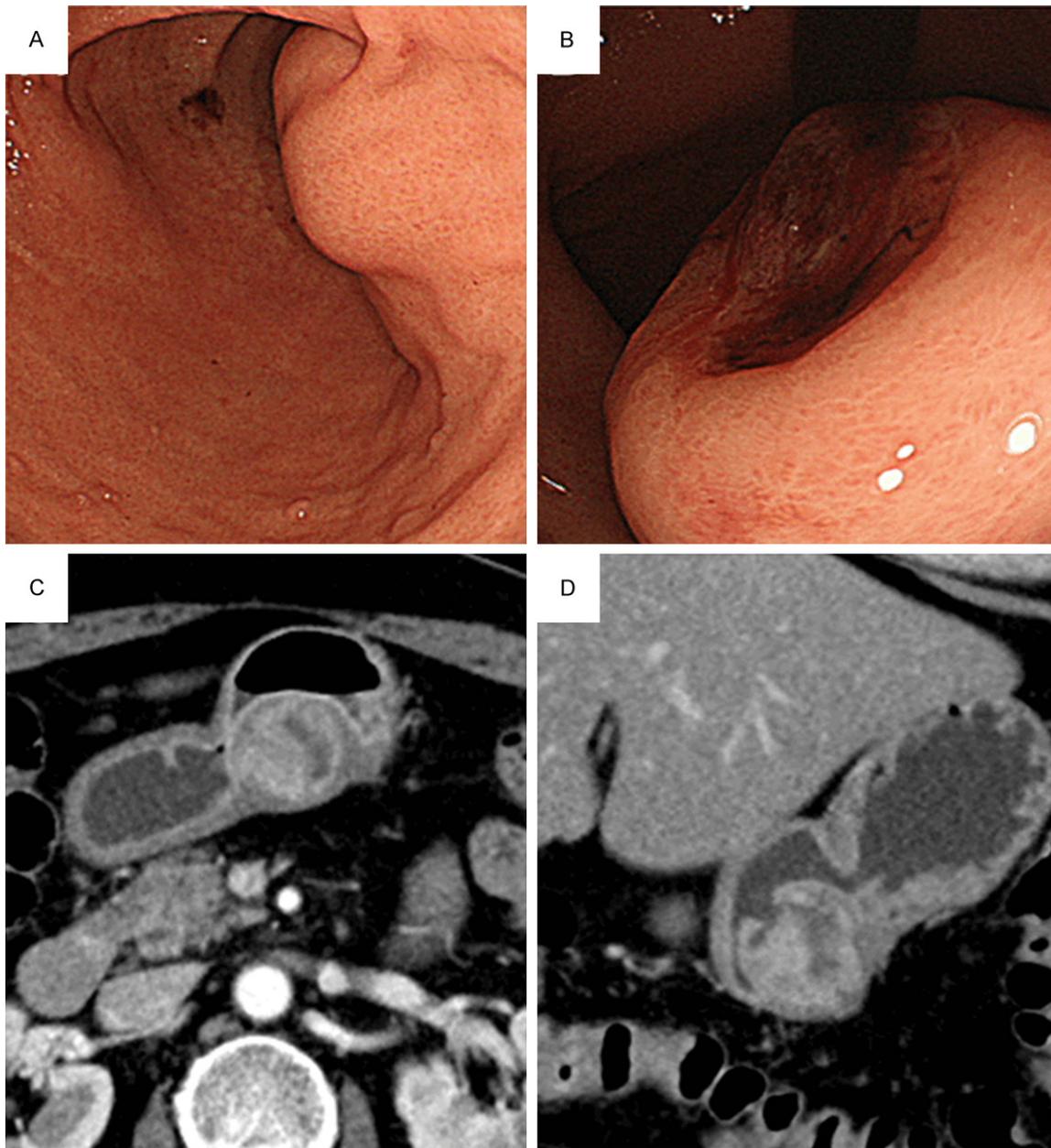


Figure 1. Upper gastrointestinal endoscopy (A, B) and contrast-enhanced computed tomography (C, D) findings. (A, B) A smooth submucosal tumor with a delle at the top. The tumor was accompanied by a small amount of blood coagulation. (C, D) (C) Axial; (D) Coronal. A round, well-margined tumor measuring 3 cm in the maximal diameter at the gastric angle showing strong contrast enhancement.

Herein we present a case of a 59-year-old woman with myxoid epithelioid GIST of the stomach showing complete lack of c-kit expression and accompanying infiltration not only of mast cells but also of abundant eosinophils. Mutational analyses revealed a *PDGFRA* exon 18 mutation: D842_H845del, D846N. Combined deletion and substitution mutation has been reported in rare instances [9, 12], but to

the best of our knowledge, D846N has never been documented.

Clinical summary

A 59-year-old woman presented to her regular doctor complaining of melena. Upper gastrointestinal endoscopy revealed an ulcerative lesion of the stomach, and she was thus

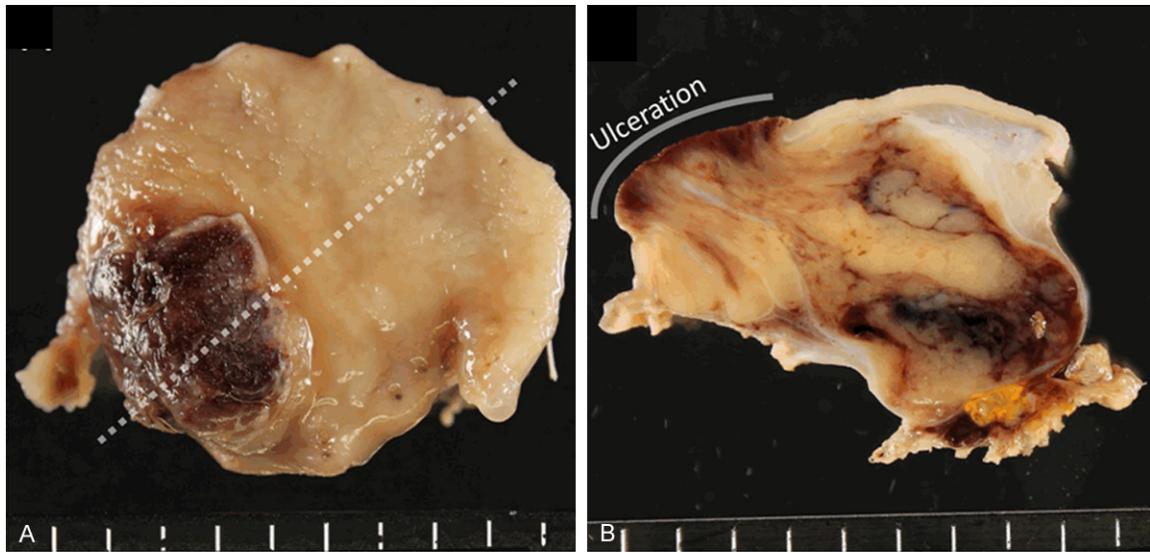


Figure 2. Gross findings of the surgically resected specimen. (A) Tumor with an ulcerated surface in the stomach. (B) The cut surface of the dotted line in (A); the tumor was mildly yellowish to tan; hemorrhagic areas were interspersed. The ulcerated surface is shown by a curvilinear line.

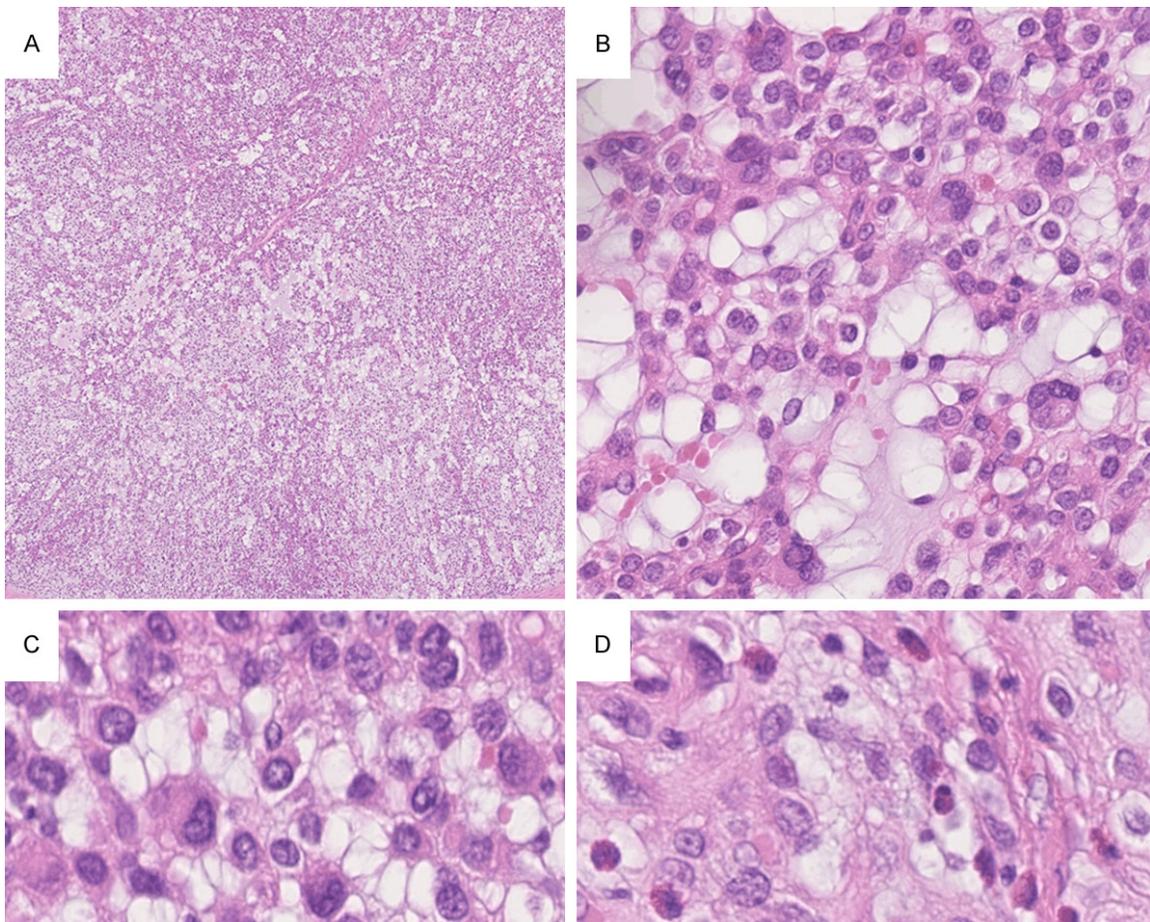


Figure 3. Microscopic findings. A. The tumor consisted of round to oval epithelioid tumor cells often showing a less cohesive pattern of growth within a myxoid background (x 20). B. The cytoplasm of the tumor cells ranged from pale to eosinophilic with mild to moderately enlarged nuclei containing small nucleoli; multinucleated tumor cells were present.

Myxoid epithelioid GIST harboring an unreported *PDGFRA* mutation

also observed (x 400). C. Some tumor cells displayed eosinophilic cytoplasm and peripherally placed nuclei, which is consistent with rhabdoid cells (x 600). D. Many eosinophils infiltrated into the tumor (x 600).

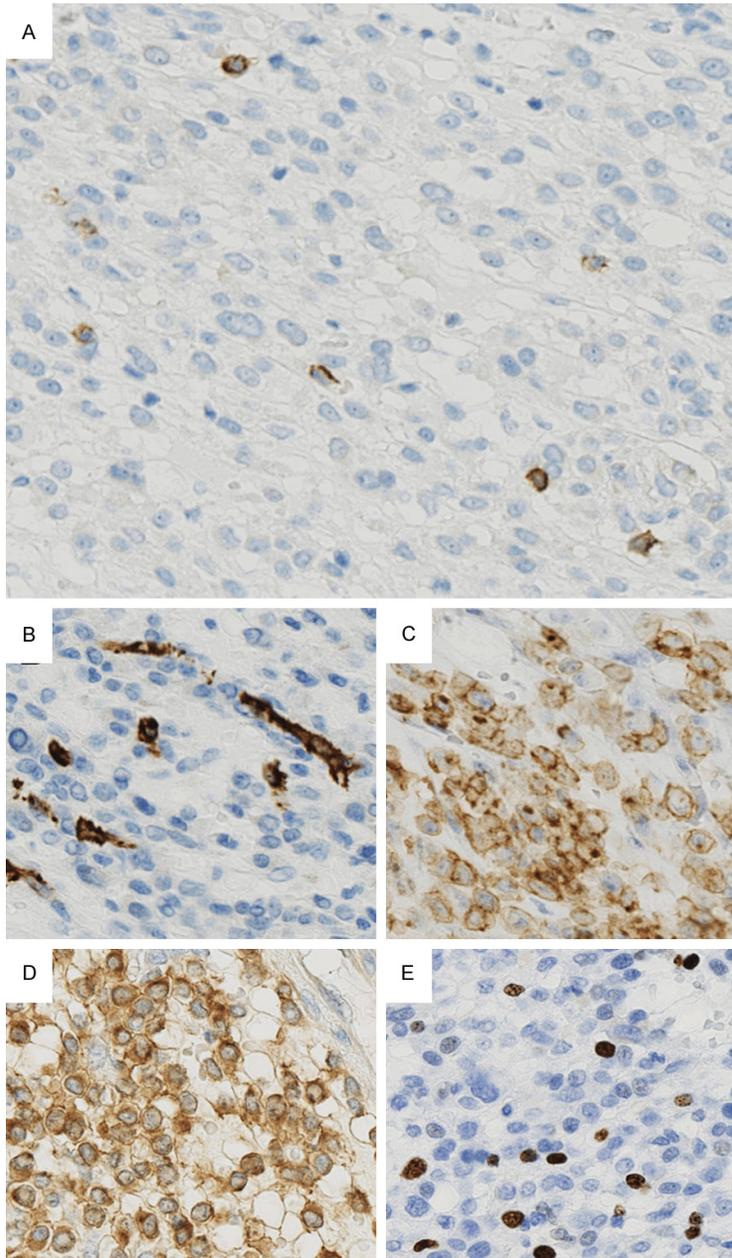


Figure 4. Immunohistochemical findings. A. Tumor cells were negative for c-kit, while mast cell infiltration was easily observed owing to their c-kit expression (x 400). B. Immunoreactivity for CD34 was not demonstrated (x 400). C. Discovered on GIST-1 accentuated the cellular membrane and Golgi apparatus of the tumor cells (x 400). D. Tumor cells strongly expressed *PDGFRA* especially in Golgi apparatus (x 400). E. Ki-67 labeling index was 16.2% (x 400).

referred to our hospital. Physical examination disclosed no remarkable findings. Laboratory

studies showed slight anemia with a hemoglobin value of 9.8 mg/dL. We performed another round of upper gastrointestinal endoscopy, during which a smooth submucosal tumor with a 'delle' was noted on the posterior wall of the gastric angle (**Figure 1A, 1B**). Abdominal contrast-enhanced computed tomography illustrated a well-margined and enhanced tumor measuring 3 cm in maximal diameter at the gastric angle (**Figure 1C, 1D**). Although GIST was strongly suspected on the basis of the clinical features, three biopsies of the tumor did not yield a specimen suitable for definitive diagnosis. As there was the need to control bleeding from the tumor in addition to making a definitive diagnosis, laparoscopic partial gastrectomy was performed. The patient's postoperative course was uneventful and she remained recurrence-free for 5 months.

Pathological findings

The surgically resected specimen revealed an ulcerated tumor (**Figure 2A**). The cut surface was mildly yellowish to tan; hemorrhagic areas were interspersed (**Figure 2B**). The size of the tumor was 42 × 28 × 22 mm.

Histopathologically, the tumor consisted of round to oval epithelioid tumor cells often showing a less cohesive pattern of growth within a myxoid background (**Figure 3A**). The cytoplasm of these cells ranged from pale to eosinophilic with mild to moderately enlarged nuclei containing small nucleoli; multinucleated tumor cells were also observed (**Figure 3B**). Some

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Myxoid epithelioid GIST harboring an unreported *PDGFRA* mutation

Table 1. Sequences of primers used in the present case

	Exon	Sequence
<i>c-kit</i>	9F	5'-ATGCTCTGCTTCTGTACTGCC-3'
	9R	5'-CAGAGCCTAACATCCCCTTA-3'
	11F	5'-CCAGAGTGCTCTAATGACTG-3'
	11R	5'-ACCCAAAAGGTGACATGGA-3'
	13F	5'-CATCAGTTTGCCAGTTGTGC-3'
	13R	5'-ACACGGCTTTACCTCAAATG-3'
	17F	5'-TGTATTCACAGAGACTTGGC-3'
	17R	5'-GGATTACATTATGAAAGTCACAGG-3'
<i>PDGFRA</i>	12F	5'-TCCAGTCACTGTGCTGCTTC-3'
	12R	5'-GCAAGGGAAAAGGGAGTCTT-3'
	18F	5'-ACCATGGATCAGCCAGTCTT-3'
	18R	5'-AAGTGTGGGAGGATGAGCCTG-3'

PDGFRA, platelet-derived growth factor receptor α ; F, forward; R, reverse.

tumor cells displayed eosinophilic cytoplasm and peripherally placed nuclei, which is consistent with rhabdoid cells (**Figure 3C**). Mitotic figures were 7 per 10 high-power fields; necrosis was absent. Eosinophils seemed to be more numerous than mast cells (**Figure 3D**). The surgical margins were free of tumor.

Upon immunohistochemistry (IHC), tumor cells were negative for c-kit (polyclonal, 1:100; Dako, Glostrup, Denmark), while mast cell infiltration was easily observed due to their c-kit expression (**Figure 4A**). Immunoreactivity for CD34 (QBEnd 10, 1:50; Dako) was not demonstrated (**Figure 4B**). Immunostaining of discovered on GIST-1 (DOG1; SP31, 1:100; Nichirei Biosciences, Tokyo, Japan) accentuated the cellular membrane and Golgi apparatus (**Figure 4C**). The tumor cells strongly expressed *PDGFRA* especially in Golgi apparatus (ab61219, 1:100; Epitomics, Burlingame, CA) (**Figure 4D**). The Ki-67 (MIB-1, 1:100; Dako) labeling index was 16.2% (162 positive tumor cells per 1000 tumor cells) (**Figure 4E**).

The diagnosis of c-kit-negative GIST was made. It was categorized in the intermediate risk group and in the moderate risk group according to the standards proposed by Fletcher et al. [14] and Miettinen et al. [15], respectively. Morphologically, this case showed the features of myxoid epithelioid GIST, which was further confirmed by c-kit negativity.

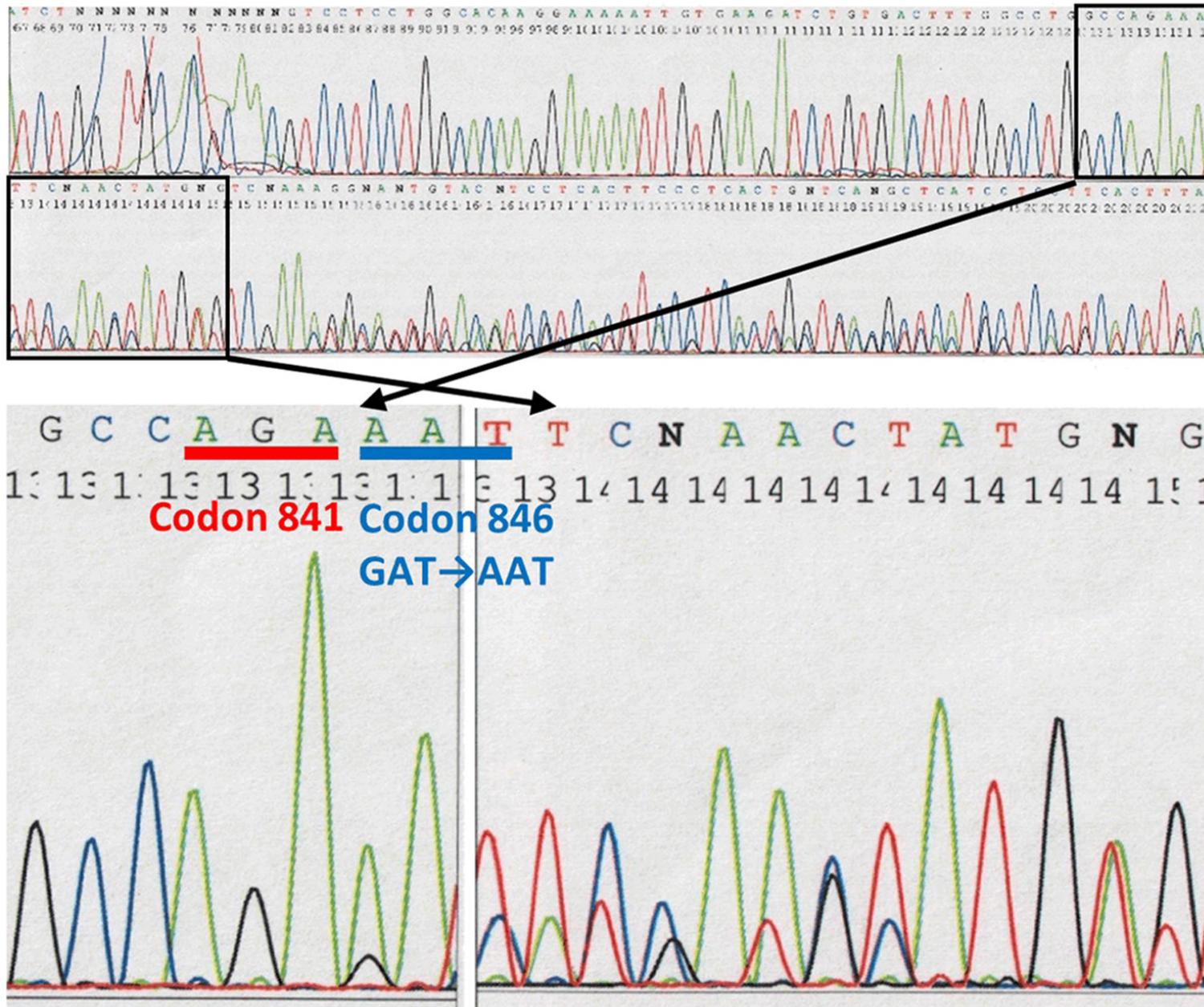
We thus performed mutational analysis of *PDGFRA* exons 12 and 18 as well as *c-kit* exons 9, 11, 13, and 17, using the polymerase chain reaction and direct sequencing methods. Mutational analyses of both *c-kit* and *PDGFRA* were performed because several cases harboring both mutations at the same time have been reported [16]. The primers used are listed in **Table 1**. The results showed *PDGFRA* exon 18 mutations consisting of deletion and substitution: D842_H845del, D846N (**Figure 5**). Other exons both in *c-kit* and in *PDGFRA* did not reveal any mutation. The presence of *PDGFRA* mutations also matched the diagnosis of myxoid epithelioid GIST.

Discussion

The most powerful predictors of *PDGFRA* mutations are the epithelioid morphology in tumor cells and tumor-infiltrating mast cells [11]. In addition, multinucleated tumor cells and rhabdoid tumor cells are relatively reliable predictors of *PDGFRA* mutations. On the other hand, the presence of myxoid stroma, which is associated with *PDGFRA* mutations as reported previously, by itself is not a good discriminating predictor between *PDGFRA*-mutated GISTs and *c-kit*-mutated ones, although myxoid changes may be striking in some cases of *PDGFRA*-mutated GISTs [11]. However, myxoid stroma in association with epithelioid tumor cells present a unique morphology, which was termed myxoid epithelioid GIST by Sakurai et al. [9]; in their report, Sakurai et al. identified that 90% (18 of 20) of the cases analyzed harbored *PDGFRA* mutations [9]. On the morphological viewpoint, mast cells are not as easy to observe as myxoid stroma and epithelioid tumor cells, and previous studies focusing on mast cell infiltration in GIST used IHC to detect this kind of infiltration [9, 11]. Therefore, myxoid and epithelioid features are an important combination recognizable on morphology alone. In the initial definition of myxoid epithelioid GIST, mast cell infiltration was not included; however, it usually accompanied this tumor subtype [9]. While the features of our case matched those of myxoid epithelioid GIST, a unique feature, eosinophil infiltration, was also noticeable.

Eosinophil infiltration as well as mast cell infiltration might be attributable to stem cell factor (SCF) expression by GIST tumor cells, expres-

Myxoid epithelioid GIST harboring an unreported *PDGFRA* mutation



Myxoid epithelioid GIST harboring an unreported *PDGFRA* mutation

Figure 5. Mutational analysis of *PDGFRA* exon 18. Substitution mutation was observed in codon 846: GAT→AAT. An in-frame deletion was observed, spanning codons 842 to 845.

sion of which in GIST was shown by Sakurai et al. [9]. SCF plays an important role in the recruitment of mast cells and eosinophils [17, 18]. As SCF is a natural ligand of the c-kit receptor [19], it is thus reasonable to infer that rapid internalization of SCF by the SCF-c-kit juxtacrine loop takes place. In c-kit-negative GISTs, this internalization would not occur, presumably resulting in mast cell infiltration [9]. Eosinophil infiltration in our case of c-kit-negative GIST would have occurred by a similar mechanism.

As concerns the diagnostic use of IHC, c-kit and CD34, which have been occasionally expressed in myxoid epithelioid GIST harboring *PDGFRA* mutations, did not show immunopositivity in our case [9]. DOG1, encoding a membrane protein associated with calcium-dependent chloride channel activity, is upregulated in GIST as revealed by gene expression profiling [20]. IHC of DOG1 has been reported to be highly specific and sensitive for GIST regardless of c-kit expression level or the underlying mutated genes [21, 22]. Expression of DOG1 was observed in our reported case as expected, given its high sensitivity even for *PDGFRA*-mutated GISTs. Some authors proposed that IHC for *PDGFRA* is a useful marker for c-kit-negative GIST, predicting *PDGFRA* mutation [23]. They showed that expression of *PDGFRA* is usually observed in GISTs and that dot-like or +/- expression is correlated with *PDGFRA* mutation in 84% (31 of 37 cases) of GISTs. In this viewpoint, the expression pattern of *PDGFRA* in our case, which highlighted Golgi apparatus by dot-like immunostaining, might be a predictor of a *PDGFRA* mutation.

Activating mutations in *PDGFRA* were reported in 35% to 67% of GISTs lacking *c-kit* mutations [3, 4]. Among *PDGFRA* mutations, the most common ones are single nucleotide substitutions, most of which have been found in exon 18; the most common substitution mutation is D842V [13]. In-frame deletions are the second most common *PDGFRA* mutations in GISTs, which tend to cluster between codons 559_572 in exon 12 and 840_848 in exon 18 [13]. A D842V mutation and deletions have been demonstrated to activate *PDGFRA* both in vitro and

in vivo [3, 4, 24]. Although the most common D842V *PDGFRA* mutation is intrinsically imatinib resistant [4, 25], approximately 30% of the *PDGFRA*-mutated GISTs other than D842V are known to be potentially imatinib sensitive [25]. Thus, imatinib would be allowed to use in our case that harbored a *PDGFRA* mutation, D842_H845del, D846N, which might be a sensitive mutation, if molecule-targeted therapy is needed in the clinical course of our patient in the future.

In conclusion, this case morphologically and immunohistochemically matched the diagnosis of myxoid epithelioid GIST. The presence of a *PDGFRA* mutation supported this diagnosis, although its mutation is a previously unreported one (exon 18: D842_H845del, D846N) to the best of our knowledge. Another unique finding in this case is eosinophil infiltration probably more abundant than mast cells. As mast cell infiltration is usually found in myxoid epithelioid GIST and mast cells and eosinophils are recruited by SCF, eosinophil infiltration in this case would have occurred by a similar mechanism to that of mast cell infiltration.

Disclosure of conflict of interest

None.

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Myxoid epithelioid GIST harboring an unreported *PDGFRA* mutation

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Myxoid epithelioid GIST harboring an unreported *PDGFRA* mutation

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