Clinical Case Report

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Gastrointestinal stromal tumor of unusual phenotype after imatinib treatment

A case report and diagnostic utility of ETV1 mRNA in situ hybridization

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Abstract

Rationale: Gastrointestinal stromal tumor (GIST) is the most common tumor of mesenchymal origin in gastrointestinal tract. Immunohistochemical (IHC) staining combined with a typical morphology is used for the diagnosis of GIST. Typically, IHC staining for v-kit Hardy–Zuckerman 4 feline sarcoma viral oncogene (KIT) and discovered on GIST-1(DOG1) is positive in almost all GISTs. However, imatinib mesylate, a specific inhibitor of KIT tyrosine kinase, frequently involves changes in the morphology and IHC staining of GIST, impeding the diagnosis. Recently, in situ hybridization (ISH) for E26 transformation-specific sequence variant 1 (ETV1) mRNA was introduced as a useful marker to diagnose GIST.

Patient concerns: We report 2 cases of gastric GIST, which expressed unusual phenotypes after imatinib therapy.

Diagnoses: The first patient was found to have a gastric subepithelial tumor in gastroduodenoscopy done for regular checkup. In biopsy of the tumor, it showed homogenous spindle cells that were positive to standard IHC markers for GIST. The second patient visited our hospital because of a palpable mass in the abdomen. In abdominal computed tomography (CT), a tumor arising from the stomach was found. A needle biopsy was done and the patient was diagnosed of gastric GIST because the biopsy showed spindle cells positive to typical IHC markers for GIST. After imatinib treatment, in both patients, the resected tumors were composed of heterogeneous spindle cells negative to KIT, DOG1, and CD34 IHC staining, which was unusual for GIST. However, ISH for ETV1 mRNA done for both biopsied and resected tumors was positive, even after imatinib treatment. A molecular analysis found a mutation in exon 11 of KIT gene before and after imatinib therapy in both patients, confirming the diagnosis of GIST.

Interventions: Both patients took neoadjuvant imatinib treatment, and afterwards, underwent a surgical resection.

Outcomes: The patients remain on imatinib treatment and no progression or recurrence has been detected to date.

Lessons: ISH for ETV1 mRNA is a useful technique in diagnosing GIST when IHC with KIT, DOG1, or CD34 fail to stain positive after imatinib therapy.

Abbreviations: CD = cluster of differentiation, CT = computer tomography, DNA = deoxyribonucleic acid, DOG1 = discovered on GIST-1, EGD = endoscopic gastroduodenoscopy, ETV1 = E26 transformation-specific sequence variant 1, GIST = gastrointestinal stromal tumor, H&E = hematoxylin and eosin, HPF = high-power field, IHC = immunohistochemistry, ISH = in situ hybridization, KIT = v-kit Hardy–Zuckerman 4 feline sarcoma viral oncogene, PCR = polymerase chain reaction, PKCθ = Protein kinase C-θ, RNA = ribonucleic acid, SMA = smooth muscle actin.

Keywords: diagnosis, ETV1, gastrointestinal stromal tumors, imatinib mesylate, immunohistochemistry, in situ hybridization

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1. Introduction

Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of gastrointestinal tract.[1] After mutations of *KIT* gene were found in GISTs,[2] it has been widely accepted that the activating mutations of KIT do a significant role in GIST pathogenesis and immunohistochemical (IHC) staining for KIT is a reliable method in diagnosing GIST.[3] In the clinical setting, diagnosis of GIST is based on the morphology and a group of IHC markers including DOG1, platelet-derived growth factor receptor-alpha (PDGFRα), CD34 as well as KIT.[3–5] A direct sequencing of KIT is sometimes necessary in diagnosis especially when KIT IHC staining is inconclusive.[6,7] It is important to understand that KIT IHC, a gold standard test, can be obscure after imatinib treatment. In such cases, the diagnosis is largely based on histopathologic and molecular analyses.[5,8–10] Recently, in situ hybridization (ISH) of ETV1 mRNA was introduced as a useful technique in diagnosis of GIST, which showed similar specificity and slightly lower sensitivity to KIT IHC staining.[4] However, there has been no attempt to identify the expression of ETV1 mRNA in the GIST after imatinib therapy, to our knowledge.

The present study reports 2 cases of GIST, which showed phenotypic change after imatinib treatment. Standard GIST markers, including KIT, DOG1, and CD34, became negative. However, ISH for ETV1 mRNA maintained its positivity in both cases after imatinib treatment.

2. Case report

2.1. Case 1

A 72-year-old male got an endoscopic gastroduodenoscopy (EGD) for a routine check-up that found a 1.5 cm sized subepithelial mass at the fundus of the stomach. The size did not increase in the EGD after 1 year. However, after 2 years, he had melena and a 7 cm sized ulcerofungating mass was found at the same site of the stomach by the EGD and abdominal CT. A hepatic mass about 3 cm in diameter was also found, suggesting a metastasis. An endoscopic biopsy of the gastric mass revealed a spindle cell tumor with minimal pleomorphism. No mitosis was found in the whole tissue, which was less than 5 high-power field (HPF) (Fig. 1A). The neoplastic cells were positive to KIT, DOG1,

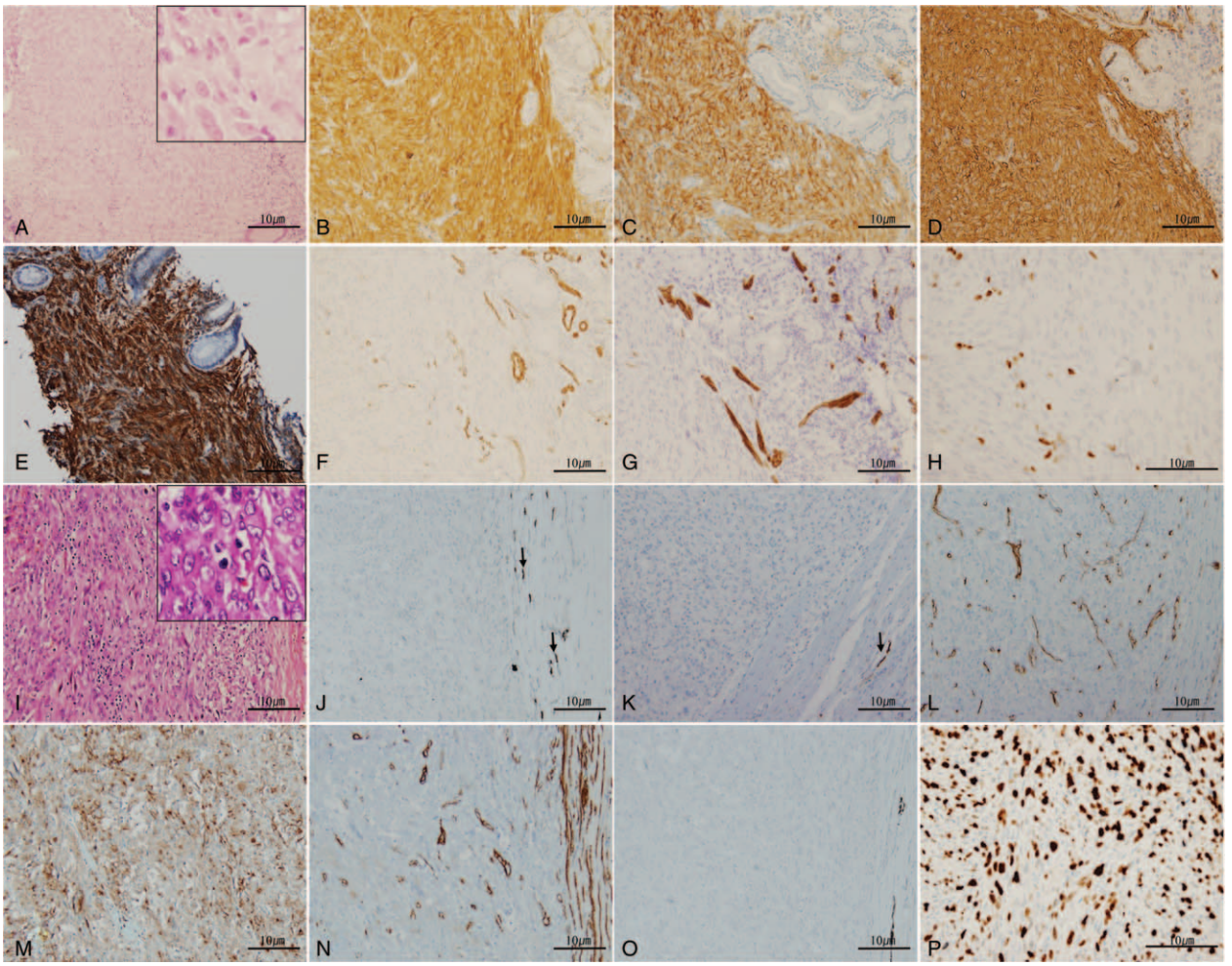

Figure 1. In the case 1, biopsy specimen shows homogenous spindle cells in H&E staining (A, ×100, ×400 in inlet). The cells are positive for KIT (B), DOG1 (C), CD34 (D), and PDGFRα (cytoplasmic and membranous) (E). IHC staining for SMA (F) and desmin (G) is negative (B–G, ×100). Ki-67 staining is positive in 5% of the tumor cells (H, ×400). After imatinib, the tumor shows spindle and epithelioid cells with moderate pleomorphism (I, H&E, ×100, ×400 in inlet). In IHC staining, the tumor cells are negative to KIT (J), DOG1 (K), and CD34 (L) but positive to PDGFRα (membranous) (M). IHC for SMA (N) and desmin (O) remains negative (J-O, ×100). Of note, submucosal plexus (J and K, arrow) and intratumoral blood vessels (L) work as an internal positive control. Ki-67 staining is positive in 5% of the tumor cells (P, ×400).

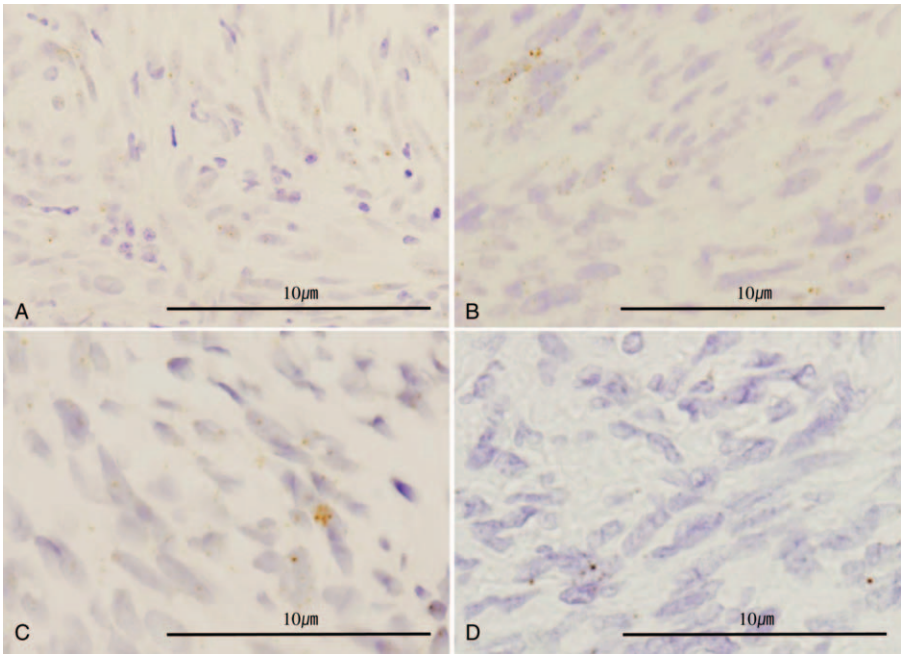

Figure 2. ISH for ETV1 mRNA shows positive signals in the nuclei of tumor cells in case 1 before (A) and after (B) imatinib treatment (×600). The second case also shows positive hybridized signals to ETV1 mRNA before (C) and after (D) imatinib (×600).

and CD34. PDGFRα was positive in cytoplasm and membrane of tumor cells. Smooth muscle actin (SMA), desmin (Fig. 1B–G), and S-100 (data not shown) were negative in IHC analysis. Ki-67 staining was positive in 5% of the tumor cells (Fig. 1H). ISH for ETV1 mRNA showed positive nuclear staining only in GIST cells (Fig. 2A). A molecular analysis for KIT gave a deletion and insertion mutation in exon 11 (see Figure, Supplemental Figure, http://links.lww.com/MD/B997, "A" demonstrates the Sanger sequencing showing the mutation in the exon 11 of *KIT* gene). Being diagnosed with GIST, the patient began to take 400 mg/day of imatinib and subsequently increased the dose to 600 mg/day because a new metastatic lesion appeared in another segment of the liver a month later. A regular workup using abdominal CT showed partial response, a decrease of the size of the gastric GIST from 7 to 3.5 cm in diameter. He was stable for 5 years, but eventually, the tumor progressed radiologically. A palliative wedge resection of the stomach revealed the tumor reaching 6.5 cm was composed of epithelioid and spindle cells with moderate pleomorphism (Fig. 1I). The mitotic activity was as high as 53/50 HPF. Among IHC markers positive in the biopsy, KIT, DOG,1 and CD34 turned negative, except for PDGFRα, which showed membranous positivity (Fig. 1J–M). Immunostains for SMA and desmin were negative (Fig. 1N–O) and S-100 was focally positive (data not shown) in tumor cells. Ki-67 staining was positive in 5% of the tumor cells (Fig. 1P). ISH for ETV1 mRNA showed diffuse positive signals on tumor cells (Fig. 2B). KIT sequencing gave the same result to the biopsy (see Figure, Supplemental Figure, http://links.lww.com/MD/B997, "B" demonstrates the same mutation to the biopsy in the surgical specimen). After the surgery, the patient increased the imatinib dose to 800 mg/day and is stable on disease for 1 year until present.

2.2. Case 2

The second patient, a 67-year-old female, visited our hospital because of a palpable mass in the abdomen. In abdominal CT, there was a 10 cm-sized mass in the stomach abutting on the pancreas and the descending colon. Gastric GIST invading to nearby organs was suspected and a needle biopsy was done. On H&E staining, the tumor was composed of atypical spindle cells and the mitosis was counted as 25/50 HPF (Fig. 3A). On IHC staining, KIT, DOG1, and CD34 were positive, but S-100, desmin, and SMA were negative (Fig. 3B–G). Ki-67 was positive in 3% of the tumor cells (Fig. 3H). PDGFRα immunostaining was unavailable because there was no remaining tissue. ISH for ETV1 mRNA showed positive signals in the nuclei of tumor cells (Fig. 2C). A complex deletion/insertion mutation of KIT was found in exon 11 (see Figure, Supplemental Figure, http://links.lww.com/MD/B997, "C" shows the result of Sanger sequencing indicating the mutation in KIT exon 11). The patient took imatinib at the dose of 400 mg/day for 10 months; afterwards, CT scan revealed a partial response to imatinib with decrease of the tumor size to 5.7 cm. In the operation, there was no sign of invasion to surrounding organs and therefore a clear wedge resection of the tumor was carried out. In the pathologic examination, the tumor was a spindle cell neoplasm extending from submucosa to serosa of the stomach, exhibiting a low mitotic activity <1/50 HPF and a massive hyaline change (Fig. 3I). The tumor cells were negative to KIT, DOG1, CD34, S-100, desmin, and SMA in IHC (Fig. 3J–O). Less than 0.1% of tumor cells were positive to Ki-67 (Fig. 3P). IHC for PDGFRα was positive in cytoplasm and membrane of tumor cells (data not shown). Although the IHC profile was unusual for GIST, ISH of ETV1 mRNA gave positive signals in tumor cells (Fig. 2D). The same mutation to the biopsy was found in exon 11 of KIT (see Figure, Supplemental Figure, http://links.lww.com/MD/B997, "D" shows the same mutation in *KIT* gene confirming the diagnosis after imatinib). The patient had been taking 300 mg/day of imatinib for 2 years after the surgery and stopped it because of neutropenia. In regular follow-ups, there has been no evidence of disease for 4 years after the surgery until now.

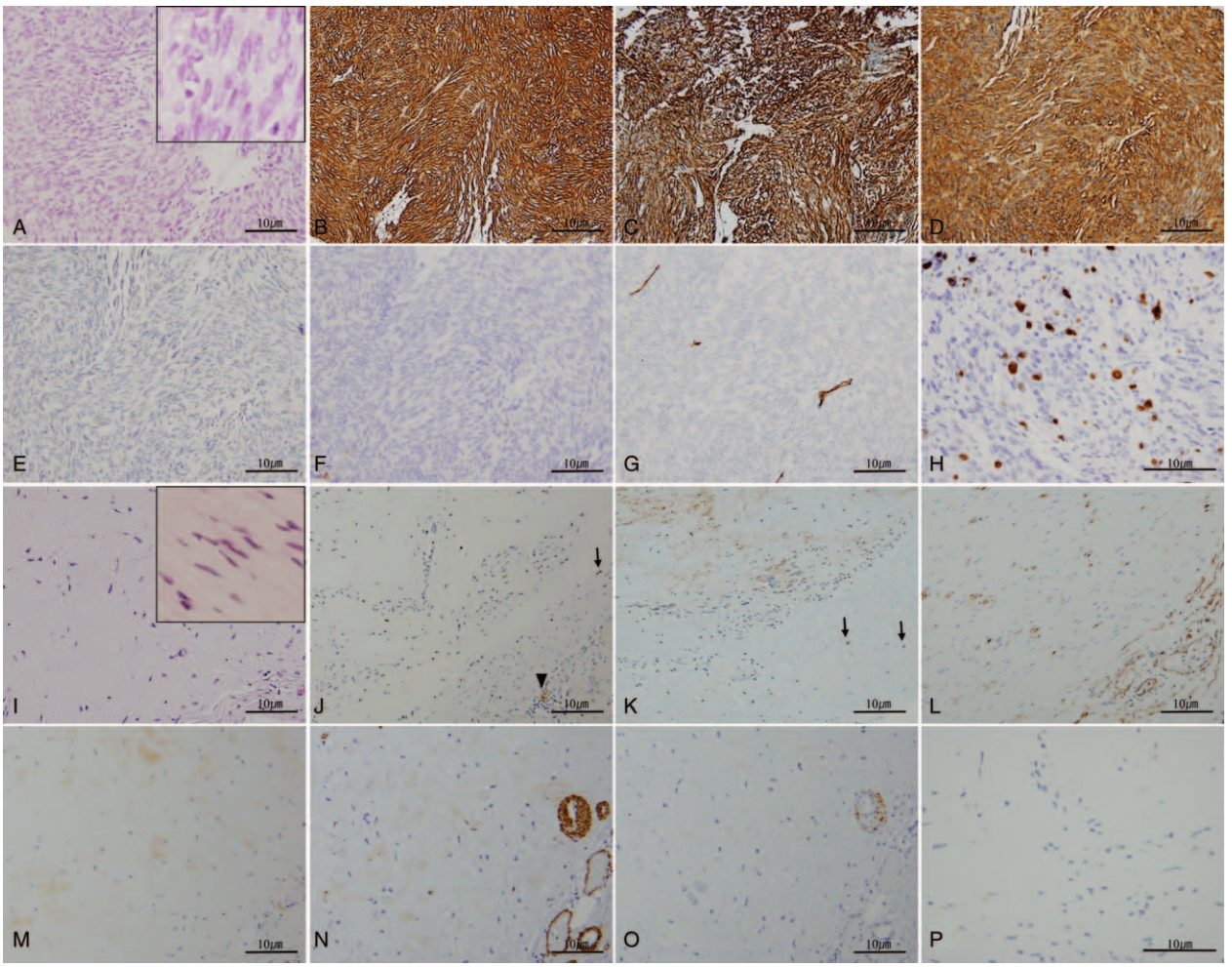

Figure 3. In the second case, biopsy showed homogenous spindle-shaped cells (A, ×100, ×400 in inlet) positive for KIT (B), DOG1 (C), CD34 (D), but negative for S-100 (E), SMA (F), and desmin (G) IHC staining (B-G, ×100). Ki-67 labelling index is 3% (H, ×400). After imatinib, the tumor consists of homogenous spindle cells with massive hyalinization (I, ×100, ×400 in inlet). IHC staining demonstrates a "null-phenotype," which is negative to KIT (J), DOG1 (K), CD34 (L), S-100 (M), SMA (N), and desmin (O) (J-O, ×100). A neuronal cell in myenteric plexus (J and K, arrow), a mast cell (J, arrow head), and endothelial cells (L) show positivity normally in each staining. Ki-67 staining is positive in less than 1% of the tumor cells (P, ×400).

3. Discussion

KIT gene located in chromosome 4q translates a receptor tyrosine kinase proteins.[11] When stem cell factor bind to this receptor, tyrosine kinase is activated and produces downstream signal pathways.[11] Mutations in this gene enable uncontrolled activation of the receptor that is associated with a survival and a proliferation of cells, which are a key mechanism of GIST pathogenesis.[3] Mutation hotspots are located in exon 9, 11, 13, and 17 of KIT.[11] Imatinib, a specific inhibitor to KIT tyrosine kinase, induces various cellular changes in GIST that demands a differential diagnosis with leiomyosarcoma or desmoid-type fibromatosis, sometimes.[9] Therefore, understanding the shift of the morphologic and IHC characteristics is a diagnostic pitfall.[5,8–10] In the first case, the patient was diagnosed with GIST of the stomach. Imatinib was prescribed, inducing a size-decrease in the tumor and maintained a stable state of disease. Nevertheless, tumor progressed in 5 years while he was taking imatinib. The tumor cells were initially spindle shaped but pleomorphic after imatinib. IHC staining showed a null-phenotype, negative to standard markers, including KIT, DOG1, and CD34. It may be ascribed to the KIT silencing effect of imatinib, which in turn, alters the common pathways of GIST pathogenesis.[9] Similarly, the GIST of the second patient demonstrated a loss of KIT, DOG1, and CD34 IHC staining after imatinib. In contrary to the first case, the second patient exhibited a good clinical response to imatinib. In patients showing a good response to imatinib, according to 1 report, cellular change with epithelioid morphology and loss of IHC was frequently seen.[8] In contrast, the cells present in the second case after imatinib retained much of the spindle morphology but lost IHC positivity. The histologic response was evident in the second patient that massive hyalinization was seen. In a case reported by Vassos et al,[5] IHC staining for KIT and DOG1 remained positive after imatinib in bland-looking cells in hyalinized stroma, suggesting that some lineages of GIST kept the original phenotype. In the second patient who was sensitive to imatinib, though, the loss of IHC staining in the spindle cells admixed with hyalinized stroma implies that the phenotypic changes do not always stem from secondary resistance to imatinib. The Ki-67 index and mitotic count were <0.1% and <1/50 HPF, respectively, in the case 2, which were higher in the case 1, 3% and 53/50 HPF, respectively.

Table 1

Histopathologic and molecular characteristics.

Case	Location	Size, cm	Operation	Mitotic count/50 HPF	Immunohistochemistry						ETV1 ISH	KIT mutation
					KIT	DOG1	CD34	SMA	Desmin	S-100		
1A	Stomach	7.0	Biopsy	0*	+	+	+	−	−	−	+	+ in exon 11 (c.1671_1676del, p. W557-V559delinsC†)
1B	Stomach	6.5	Resection	53	−	−	−	−	−	Focal+	+	+ in exon 11 (c.1671_1676del, p. W557-V559delinsC†)
2A	Stomach	10.0	Biopsy	25	+	+	+	−	−	−	+	+ in exon 11 (c.1672_1681delinsC, p. K558-E561delinsQ†)
2B	Stomach	5.7	Resection	<1	−	−	−	−	−	−	+	+ in exon 11 (c.1672_1681delinsC, p. K558-E561delinsQ†)

A, before imatinib therapy; B, after imatinib therapy; +, positive; −, negative.

CD = cluster of differentiation, DOG1 = discovered on GIST-1, ETV1 = E26 transformation-specific sequence variant 1A, HPF = high-power field, ISH = in situ hybridization, KIT = v-kit Hardy–Zuckerman 4 feline sarcoma viral oncogene, SMA = smooth muscle actin.

* The whole tissue was less than 5 field.

† Based on accession number NM_000222.2.

It is in concordant with a previous study, which indicated that a high mitotic index was seen in the patients who gained secondary resistance to imatinib.[12] Although some aberrant expressions of IHC staining such as cytokeratin, desmin, and CD31 were reported after imatinib,[5,8,9] no such expressions were found in our cases.

Diagnosing GIST is usually dependent on the IHC studies, including KIT and DOG1. However, they are not always straightforward in a small number of GIST.[4] In case of GIST negative to either KIT or DOG1, additional IHC markers are useful for diagnosis. For example, dot-like perinuclear pattern of PDGFRα IHC helps to diagnose KIT-independent GIST, although it is not routinely used because DOG1 is usually positive (98%) in KIT-negative PDGFRα-mutant GIST.[13] Because cases presented here harbored KIT mutations and PDGFRα IHC stained in cytoplasm and membrane of tumor cells nonspecifically, PDGFRα IHC did not aid in the diagnosis of our cases.[13] In addition, Protein kinase C-θ (PKCθ), a regulatory factor to KIT, is positively correlated to KIT expression.[4] IHC for PKCθ was positive in 6 of 6 KIT-negative GIST and even positive in some of KIT/DOG1 double-negative cases.[14] However, it appeared that the specificity of PKCθ IHC for diagnosing GIST is relatively low.[4] Furthermore, with regard to imatinib-induced IHC-negative GIST, the usability of additional IHC markers is still in question.

As manifested in our cases, the identification of mutations in KIT or PDGFRα is a diagnostic clue when the morphology and the IHC staining do not support GIST, which is often related to imatinib.[5,6,10–12] Secondary mutations are often identified in patients who got secondary resistance to imatinib, most commonly in exon 13 of KIT.[10,12] Loss of heterozygosity or gene amplification of KIT can induce the secondary resistance too.[9] Nonetheless, the secondary mutations were not always identified in IHC-negative cases after imatinib,[12] in agreement with our results. KIT-independent oncogenic pathway may have been activated after the usage of imatinib, released the tumors from KIT dependency, exceeded the oncogenic role of KIT, and shook off the typical IHC expressions of GIST including KIT and DOG1 in the case 1 and 2.[8,9]

ETV1, a variant of ETS family members, cooperates with KIT in oncogenesis of GIST, which differs from other ETS-dependent malignancies such as prostate cancer, melanoma, and Ewing sarcoma.[15] A high and universal expression of ETV1 in GIST cells was verified in experiments[15] and was applied in clinical diagnosis that yielded 95% of sensitivity and specificity.[4] For example, 387 of 407 GIST cases of various origins were positive to ETV1 mRNA ISH.[4] In addition, expression of ETV1 mRNA was specific to GIST cells and absent in normal tissue, such as epithelium, muscle, and blood vessel in our study. ISH for ETV1 mRNA was positive in the present cases even though IHC staining for KIT and DOG1 changed to negative after imatinib treatment, reiterating its diagnostic utility for KIT/DOG-negative GIST.[4] Importantly, KIT-inhibition by imatinib induced loss of ETV1 protein but did not affect ETV1 mRNA level.[15] IHC for ETV1 was positive only about 50% of GIST patients and it was significantly lower after imatinib treatment.[16] Therefore, ISH for ETV1 mRNA has a distinct diagnostic value for GIST after imatinib therapy. We need to keep in mind the fact that about 5% of gastrointestinal non-GIST mesenchymal tumors tested were positive to ISH for ETV1 mRNA, which included leiomyosarcoma and malignant peripheral nerve sheath tumor,[4] and should consider comprehensive pathologic features to make correct diagnosis. ETV1 mRNA expression is not interfered in by secondary resistance to imatinib as discussed in the case 1, although it needs to be further evaluated. Finally, ETV1 may have a therapeutic and prognostic implications for GIST patients.[16] Because ETV1 is regulated by KIT and is enriched in KIT-positive GIST,[4,15,16] its role as a biomarker to tyrosine kinase inhibitor treatment is clinically intriguing but largely unrevealed.

In summary, we report 2 cases of GIST whose phenotypes changed after imatinib treatment (Table 1). The first patient developed secondary resistance, whereas the second responded well to imatinib. At the time of diagnosis, the biopsies revealed spindle cell histology with typical IHC results for the GIST. After imatinib treatment, the tumors showed much more pleomorphic morphology. In addition, the IHC staining revealed unusual phenotypes, negativities to KIT, DOG1, and CD34. Molecular analyses helped the diagnosis after imatinib treatment by revealing mutations in *KIT* gene, which were same to those of biopsied tumors. ISH for ETV1 mRNA was constantly positive to the tumor cells pre- and post-imatinib, regardless of secondary resistance to imatinib. Therefore, ISH technique of ETV1 mRNA is diagnostically useful, especially for GIST of unusual phenotype after imatinib treatment.

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