World Journal of Gastrointestinal Oncology

Online Submissions: http://www.wjgnet.com/1948-5204office
wjgo@wjgnet.com
doi:10.4251/wjgo.v2.i9.364

World J Gastrointest Oncol 2010 September 15; 2(9): 364-368
ISSN 1948-5204 (online)

CASE REPORT

Immunohistochemical and molecular genetic analyses of multiple sporadic gastrointestinal stromal tumors

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Received: April 7, 2010 Revised: August 3, 2010
Accepted: August 10, 2010
Published online: September 15, 2010

Abstract

A 77-year-old Japanese male patient was admitted to our hospital complaining of general fatigue and melena. A gastroduodenal endoscopic examination revealed no definitive localized lesions. However, both a large amount of cruor and blood flow from the small intestine into the ascending colon was observed during the colonoscopic examination. At least three tumors, believed to originate from the small intestine, were detected by abdominal computed tomography. Based on these findings, multiple and hemorrhagic small intestinal tumors were diagnosed and surgical treatment of the tumors planned. During the celiotomy, twelve tumors were found in the small intestine. Intestinal wedge or partial resection was applied. All excised specimens demonstrated morphology of a submucosal tumor and the largest tumor had a delle with coagulation on the mucosal face. In the histological findings, hematoxylin and eosin staining showed spindle cell morphology. The immunohistochemical examination revealed that the tumor cells were diffusely positive for KIT and CD34. The myenteric plexus layer of the small intestine was focal-positive for KIT and showed no intestinal cells of Cajal hyperplasia. The tumor sequencing results revealed an identical missense mutation in codon 642 of *c-kit* exon 13 leading to the replacement of lysine by glutamic acid and a silent germ-line mutation in exon 12 of the *PDGFRA* gene concerning whole blood, normal mucosa and tumors. We concluded that the current subject was categorized as having multiple sporadic-type gastrointestinal stromal tumor with identical mutational types. Although the patient did not receive any adjuvant chemotherapy, there has been no sign of recurrence over the 3 years since the surgery.

Key words: Gastrointestinal stromal tumor; Platelet-derived growth factor receptor a; K642E; *c-kit*; Missense mutation; Germline mutation; KIT; Surgery

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Hiraki M, Kitajima Y, Ohtsuka T, Kai K, Miyake S, Koga Y, Mori D, Noshiro H, Tokunaga O, Miyazaki K. Immunohistochemical and molecular genetic analyses of multiple sporadic gastrointestinal stromal tumors. *World J Gastrointest Oncol* 2010; 2(9): 364-368 Available from: URL: http://www.wjgnet.com/1948-5204/full/v2/i9/364.htm DOI: http://dx.doi.org/10.4251/wjgo.v2.i9.364

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most com-

mon mesenchymal tumors of the gastrointestinal tract, and are derived from the interstinal cells of Cajal (ICCs)[1]. Somatic mutations in the *c-kit* or platelet derived growth factor receptor A (*PDGFRA*) genes are frequently found in most GISTs, and are recognized as gatekeeper molecular events[1-3]. GIST generally occurs as a sporadic solitary neoplasm whereas the occurrence of multiple primary neoplasms is considered to be an exceptional event, restricted to Carney's syndrome[4], pediatric GISTs[5], type 1 neurofibromatosis (NF1)-associated GISTs[6-8], familial GISTs[9,10] and multiple sporadic GISTs[10-12]. We herein report a case of twelve simultaneous sporadic GISTs in the small intestine that were successfully treated with surgical resection and were immunohistochemically examined and genetically analyzed the *c-kit* and *PDGFRA* mutations.

CASE REPORT

A 77-year-old Japanese male patient was admitted to our hospital complaining of general fatigue and melena. The physical examination revealed no remarkable abnormalities except for anemia in the conjunctiva. There was no notable family history. Diabetes mellitus and benign prostatic hyperplasia were concomitant illnesses in the patient. Laboratory studies performed at the time of patient admission showed anemia (hemoglobin, 6.8 g/dL, hematocrit, 19.7%), elevation of the blood urea nitrogen (38.3 mg/dL) and a decrease in the total protein and albumin (5.0 g/dL, 3.5 g/dL respectively). Because hemorrhage from an alimentary tract was strongly suspected, an upper gastrointestinal endoscopy and colonoscopy were performed. A gastroduodenal endoscopic examination revealed no definitive localized lesions. However, both a large amount of cruor and blood flow from the small intestine into the ascending colon was observed during the colonoscopic examination. At least three tumors, believed to originate from the small intestine, were detected by abdominal computed tomography (CT) (Figure 1). Based on these findings, multiple and hemorrhagic small intestinal tumors were preoperatively diagnosed and surgical treatment of the tumors planned. During the celiotomy, twelve tumors were found in the small intestine (Figure 2A); the distances from the Treitz' ligament were 20, 50, 100, 150, 170, 180, 200, 220, 240, 290, 295 and 300 cm respectively (Table 1). The sizes of the tumors ranged from 2 to 70 mm in diameter (Table 1). No other lesions were found in other organs. Intestinal wedge or partial resection was applied (Table 1) and the remnant small intestine was 170 cm in length. All excised specimens demonstrated morphology of a submucosal tumor and the largest tumor had a delle with coagulation on the mucosal face (Figure 2B). In the histological findings, hematoxylin and eosin staining showed spindle cell morphology (Figure 3A). The mitotic count in all the tumors was less than 3 per 50 high-power fields. The MIB-1 index was less than 2%. The immunohistochemical examination revealed that the tumor cells were diffusely positive for KIT (Figure 3B) and CD34, focal-positive for SMA and S-100 and negative for desmin. The myenteric plexus layer of the small intestine was focal-positive for KIT and showed no ICCs hyperplasia. According to the mitotic count and tumor size,

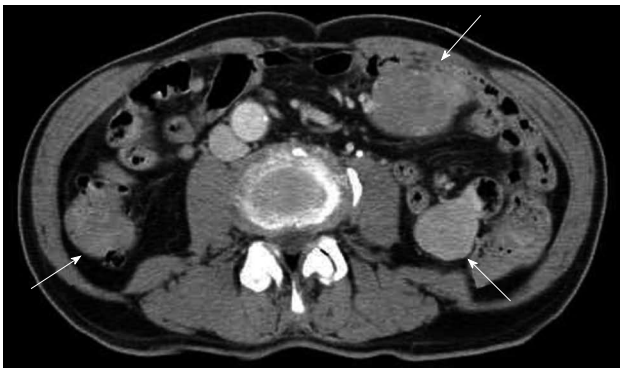

Figure 1 Computed tomography revealed multiple tumors in the abdominal cavity (arrows).

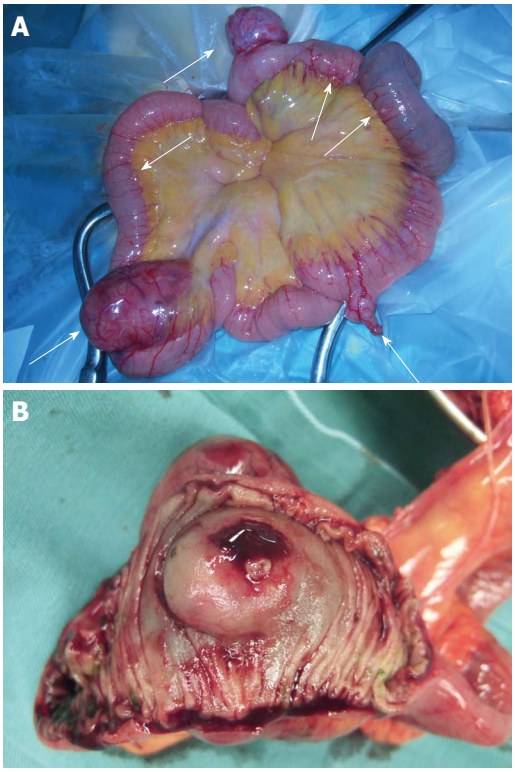

Figure 2 Intraoperative findings showed the multiple submucosal tumors in the small intestine (arrows: A) and the largest resected tumor revealed the delle with ulceration and cruor (B).

the present patient was finally diagnosed as having multiple GISTs with an intermediate risk for aggressive behavior (Table 1)[13]. The mutation analysis was performed in relation with exons 9, 11, 13 and 17 of the *c-kit* gene and exons 12, 14 and 18 of the *PDGFRA* gene in the whole blood, normal intestinal mucosa, the largest (tumor #10) and the second largest tumor (tumor #5) using polymerase chain reaction (PCR) and direct sequencing of the PCR products as previously described[10, 11]. The tumor sequencing results revealed an identical missense mutation in exon 13 of the *c-kit* gene and a silent germ-line mutation in exon 12 of the *PDGFRA* gene concerning whole blood, normal mucosa and tumors (Figure 4, Table 2). The patient's course was uneventful after surgery and he was discharged from the hospital on the 8th postoperative day. Although the patient

Table 1 Details of the twelve gastrointestinal stromal tumors

Tumor number	Localization, distance from treitz ligament (cm)[a]	Tumor size (mm)	Resection	Mitotic counts	Risk stratification
#1	20	5 × 5	Wedge resection	< 5/50 HPF	Very low risk
#2	50	3 × 3	Wedge resection	< 5/50 HPF	Very low risk
#3	100	10 × 10	Wedge resection	< 5/50 HPF	Very low risk
#4	150	2 × 2	Wedge resection	< 5/50 HPF	Very low risk
#5	170	50 × 40	Partial resection	< 5/50 HPF	Intermediate risk
#6	180	10 × 10	Partial resection	< 5/50 HPF	Very low risk
#7	200	4 × 4	Partial resection	< 5/50 HPF	Very low risk
#8	220	5 × 5	Partial resection	< 5/50 HPF	Very low risk
#9	240	30 × 20	Partial resection	< 5/50 HPF	Low risk
#10	290	70 × 60	Partial resection	< 5/50 HPF	Intermediate risk
#11	295	5 × 5	Partial resection	< 5/50 HPF	Very low risk
#12	300	6 × 6	Partial resection	< 5/50 HPF	Very low risk

[a]The full length of the small intestine was 410 cm, HPF: High-power field. The tumor number #5 to #12 are resected together.

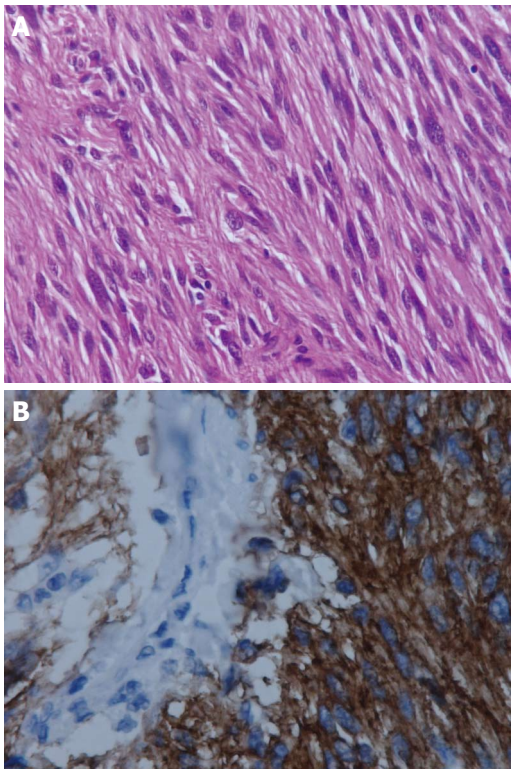

Figure 3 Histological findings and immunohistochemical examination. A: Hematoxylin and eosin staining showed a spindle-cell morphology (original magnification 200 ×), B: An immunohistochemical examination revealed that the tumor cells were diffuse positive for KIT (original magnification 200 ×).

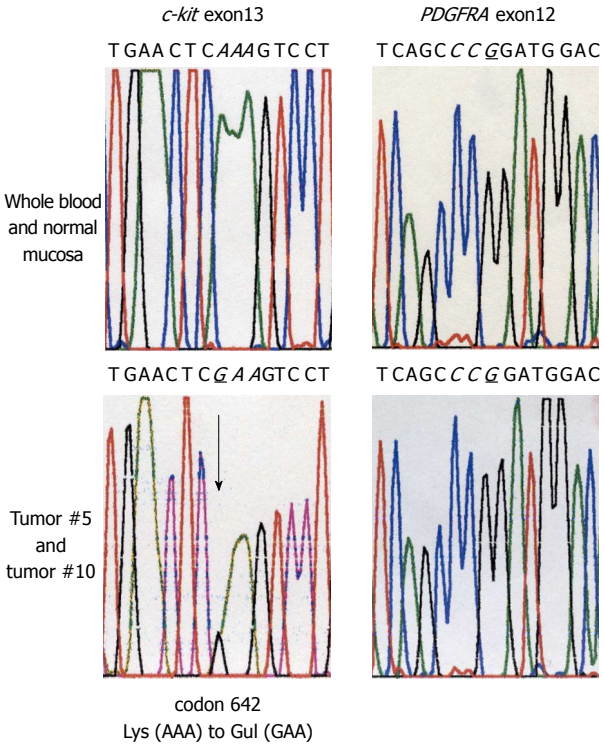

Figure 4 Sequence analysis of the *c-kit* exon 13 and Platelet derived growth factor receptor A exon 12. *PDGFRA:* Platelet derived growth factor receptor A.

did not receive any adjuvant chemotherapy, there has been no sign of recurrence over the 3 years since the surgery. The family including sibling and children and relatives did not exhibit GIST.

DISCUSSION

Most sporadic GISTs occur as solitary lesions and multiple tumors are extremely rare. Multifocal GISTs in adults are classified as sporadic with distinct or identical mutations, familial GISTs, NF1-associated GISTs and metastatic disease, according to the clinical features, family history, histology, immunohistochemical studies and mutation patterns (Table 3)[10,12]. In the present case, the discrimination of the multiple GISTs was considered as follows.

Concerning clinical features, patients with NF-1 are generally characterized as having systemic pigmented skin spots, skin and subcutaneous multiple neurofibromas and Lisch nodules[6-8]. Familial GIST is also characterized by hyperpigmentation, urticaria pigmentosa and dysphagia even though a small number of cases did not demonstrate any symptoms[9]. The present case did not show any symptoms as mentioned above. In addition, the family and relatives did not exhibit corresponding symptoms. Thus, we concluded that the present case was not categorized as familial GIST.

In the case of multiple tumors in the abdomen, peritoneal disseminated metastasis is a very common differential

Table 2 Result of the deoxyribonucleic acid sequence

Sample	*c-kit*				*PDGFRA*		
	Exon 9	Exon 11	Exon 13	Exon 17	Exon 12	Exon 14	Exon 18
Whole blood	WT	WT	WT	WT	silent mutation	WT	WT
Normal mucosa	WT	WT	WT	WT	silent mutation	WT	WT
Tumor #5	WT	WT	missense mutation	WT	silent mutation	WT	WT
Tumor #10	WT	WT	missense mutation	WT	silent mutation	WT	WT

PDGFRA: Platelet derived growth factor receptor A; WT: Wild-type.

Table 3 Discrimination of the multiple gastrointestinal stromal tumor in adult[6-10,12]

	Clinical features	Mutation	Hyperplasia of ICC	Family history	Muscularis propria involvement
Sporadic GIST	(-)	(+) Somatic mutation	(-)	(-)	(+)
Familial GIST	(+) Skin pigmentation	(+) Germline mutation	(+) Diffuse	(+)	(+)
Neurofibromatosis type 1 associated GIST	(+) Skin nodule and pigmentation	(-) rare	(+) Focal or mild	(+)	(+)
Metastatic disease	[a]	[a]	[a]	[a]	(-)

[a]Same or similar to primary tumor. ICC: Interstitial cells of Cajal; GIST: Gastrointestinal stromal tumor.

diagnosis. The present case showed multiple tumors and all twelve tumors were located in the small intestine. The preoperative examinations, celiotomy and postoperative follow-up confirmed that there were no neoplasms in the other abdominal organs. Furthermore, the histological and immunohistochemical studies showed an overlying serosal membrane on the tumors, thus suggesting that all twelve tumors were of small intestinal origin. Based on these findings, we considered that the tumors were multiple GISTs rather than disseminated GISTs.

The immunohistochemical study of the normal tissues in the present small intestine demonstrated no ICC hyperplasia. Because multiple sporadic GISTs show no ICC hyperplasia, NF1-associated GISTs demonstrate focal or mild hyperplasia and familial GISTs reveal diffuse hyperplasia[10], the current subject was classified as having multiple sporadic GISTs.

GISTs have activating mutations of *c-kit* in exons 9, 11, 13 and 17 and exons 12, 14 and 18 of *PDGFRA*[14,15]. The most frequent mutation is identified in exon 11 of the *c-kit* gene, resulting in activation of the KIT receptor[2]. Several studies in the analysis of DNA sequence of multiple GISTs reported that sporadic GISTs showed somatic mutations[10,12] and NF1-associated GISTs had no mutations or few mutations[6-8] and familial GISTs revealed germline mutations[9]. In the present case, we identified conserved tumor missense mutations at exon 13 of *c-kit* and a silent germline mutation in exon 12 of the *PDGFRA* gene in patient whole blood, normal mucosa and tumors. The silent mutation in exon 12 of *PDGFRA* did not correspond to the known mutation site in the hot spots of this gene. In addition, this silent mutation did not result in a change to the amino acid sequence of protein. However, the missense mutation in codon 642 of *c-kit* exon 13 led to the replacement of lysine by glutamic acid (K642E). Furthermore, this mutation corresponded to the previous report by Isozaki in 2000[16]. Isozaki *et al*[16] reported two patients with familial multiple GISTs: a female patient and her son presented with 20 and 13 multiple GISTs respectively. In the sequencing analysis, the authors identified this point mutation in both tumors and normal pancreatic tissues. These results suggest that a point mutation in codon 642 of exon 13 of the *c-kit* gene may play an important role in the development of multiple GISTs. Taken together, we concluded that the current subject was categorized as having multiple sporadic-type GISTs with identical mutational types based on the physical examination, family history and the appearance of ICCs.

A surgical resection is still considered to be the standard treatment for GIST and a sufficient margin should be removed to complete the resection, even if the tumor is small[17]. Dissection of regional lymph nodes is unnecessary because GISTs rarely involve lymph node metastasis[18]. Although twelve tumors were scattered throughout the small intestine in the current case, our treatment strategy was to perform complete resection and to preserve the small intestine to prevent short-bowel syndrome. At least 30% of the normal intestinal length must be preserved for proper nutrient absorption[19,20] and we were able to preserve approximately 41.5% (170 cm) of small intestine by wedge resections and partial resection. The present patient is doing well without any loss of his body weight. In a treatment of multiple tumors of the small intestine, the length of the remnant small intestine and the preserved function must be taken into consideration. As a surgical treatment, the efficacy of the laparoscopic resection for multiple GISTs is reported[21,22]. Although we considered performing laparoscopic surgery, laparoscopic surgery is recommended when the tumor measures less than 5 cm

in diameter[21]. In addition, a subject with multiple GISTs should be evaluated and the precise number and sites of all the tumors to be resected. These careful intra-operative evaluations are sometimes difficult because a tactile sensation is lacking during laparoscopic surgery.

There is growing evidence that the adjuvant therapy of imatinib mesylate has been shown to improve the outcomes of surgical resection[23,24]. Recently, adjuvant therapy for the GIST is recommended for intermediated and especially high-risk GIST. In addition, GIST with KIT exon 11 mutation appear to be sensitive to imatinib and sunitinib has shown clinical benefit in all major GIST mutational subtypes, particularly in patients with wild-type or KIT exon 9 genotype and against GIST with secondary KIT exon 13 or 14 mutations[24]. We should have performed the adjuvant therapy in this case due to the intermediated risk. However, the adjuvant therapy for complete resection with tumor-free margin was controversial when we experienced this case in 2007. In spite of the fact that the patient did not receive adjuvant therapy, he has been well without any recurrence. Thus, further investigation or analysis of the multiple GIST, focusing not only on risk stratification but also on total number of tumor in other cases with multiple GIST, is needed.

In conclusion, we experienced a rare case with multiple sporadic GISTs in the small intestine which was successfully treated by surgical resection. The tumor characterization using the DNA sequence analysis is thus considered to be useful for categorizing multiple GISTs and careful intra-operative evaluation is important to perform the complete resection of tumors. As biological behaviors of multiple GISTs are still unknown, further investigation will be necessary.

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