Gastric Cancer (2006) 9: 235–239
DOI 10.1007/s10120-006-0368-5

Case report

Recurrent gastrointestinal stromal tumor (GIST) of the stomach associated with a novel *c-kit* mutation after imatinib treatment

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Abstract

A 57-year-old man with gastrointestinal stromal tumor (GIST) of the stomach with peritoneal dissemination underwent gastrectomy. After surgery, he was treated with 400 mg/day of imatinib, without recurrence, for 26 months. At 26 months, the imatinib dose was reduced because of nausea, and 4 months after the dose reduction, recurrence of GIST was detected, for which surgical resection was performed again. The first surgical specimen had a mutation of exon 11 in the c-kit receptor gene. Intriguingly, the second surgical specimen had a novel mutation of exon 17, in addition to the above-mentioned mutation, in the *c-kit* receptor gene. Based on the result of molecular analysis, the novel mutation of exon 17, induced by longterm chemotherapy, was judged to have been responsible for the recurrence, which perhaps was triggered by the dose reduction of imatinib.

Key words Gastrointestinal stromal tumor · Imatinib · Resistance · *c-kit* · Mutation

Introduction

Gastrointestinal stromal tumors (GISTs) account for the majority of gastrointestinal mesenchymal tumors, including leiomyosarcomas and leiomyoblastomas. Most GISTs express c-kit receptor tyrosine kinase (KIT) [1]. Although surgery used to be the only valid therapy for GISTs, imatinib mesylate, a multitargeted tyrosine kinase inhibitor, has recently been reported to be effective therapy for GIST [2]. Nevertheless, several reports to date indicate that the acquisition of *c-kit* gene mutations can result in resistance to imatinib treatment [3,4]. We report a patient who developed recurrent GIST, in association with the development of a secondary mutation in the *c-kit* receptor gene.

Offprint requests to: T. Koyama
Received: November 8, 2005 / Accepted: February 14, 2006

Case report

A 57-year-old man was referred to our hospital because of a submucosal tumor in the stomach. Upper gastrointestinal endoscopy revealed an elevated lesion, with a deep central depression, in the greater curvature of the gastric body. Biopsy specimens indicated GIST. He underwent laparotomy, in which an extramural tumor 14 cm in diameter, was found in the lower gastric body; there was also a great account of peritoneal dissemination. He underwent distal gastrectomy without lymphadenectomy. Two tumors, each approximately 5 cm, in the peritoneum were also resected. GIST in the stomach was confirmed by pathological and immunohistochemical examinations, which demonstrated an interlacing pattern of spindle cells, and positive staining for CD117 (Fig. 1) and CD34, with staining for smooth muscle actin and S-100 protein being negative.

Postoperatively, daily administration of imatinib (400 mg/day) was started, and no recurrence was observed on imaging studies done during the next 26 mos; there were no side effects during this time. However, at 26 months, the dose had to be reduced to 200 mg per day, because of gastrointestinal toxicity (nausea). Four months after the first dose reduction, intraabdominal recurrence was suspected, shown by computed tomography (CT; Fig. 2B).

The dose of imatinib was therefore gradually increased, back to 400 mg per day. However, the recurrent tumor continued to grow, and he underwent reoperation. Peritoneal dissemination was not observed, and resection of the remnant tumor was carried out with combined colonectomy. After this operation, imatinib treatment was again started, and there has been no recurrence for the 7 months since the second operation.

Mutations of the *KIT* receptor and platelet-derived growth factor receptor alpha (*PDGFRA*) genes were

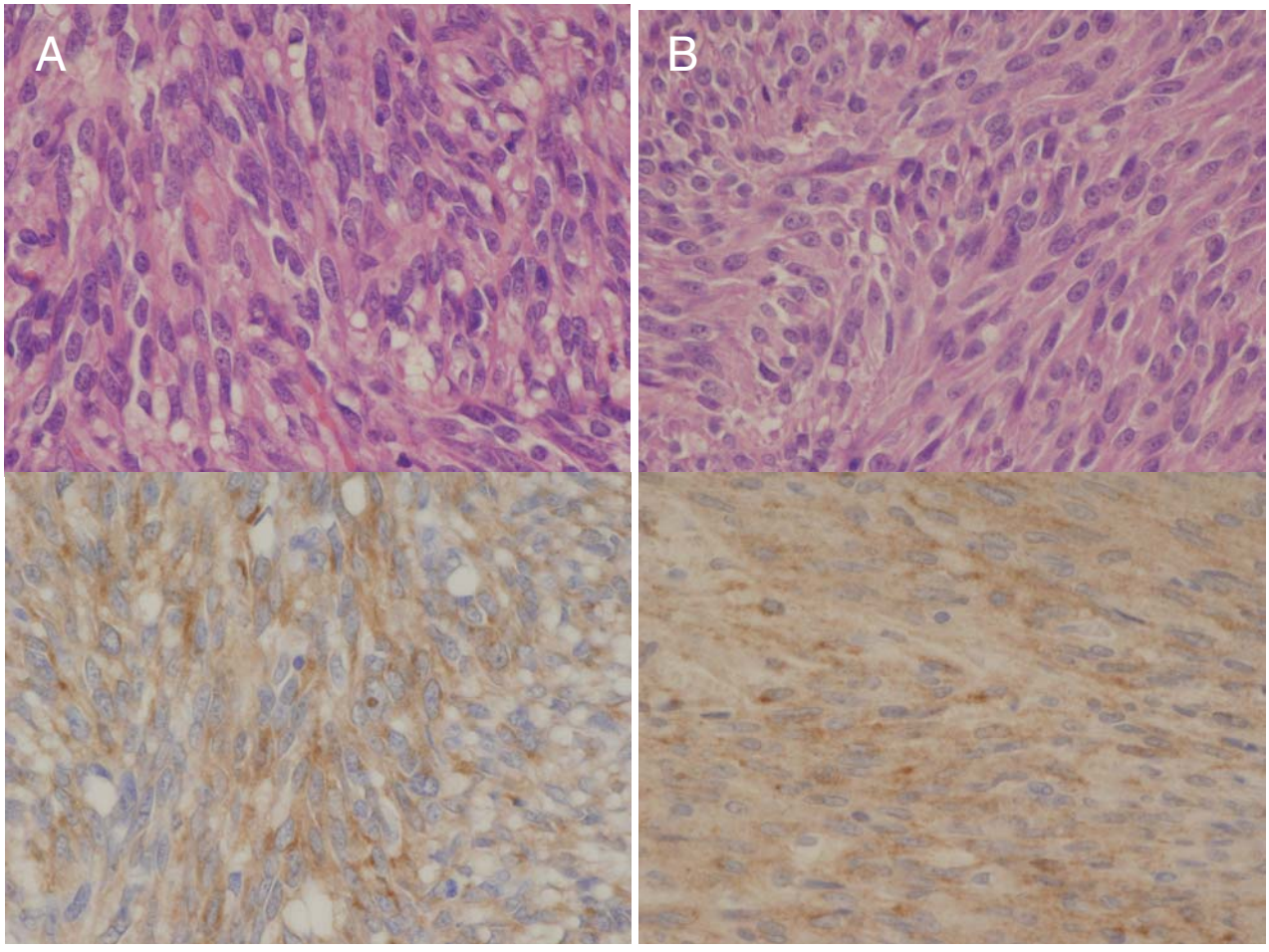

Fig. 1A,B. Histological appearance of the surgical specimens of **A** the first and **B** the second operation. The pathological finding showed an interlacing pattern of spindle cells. Both specimens were positive for KIT. *Upper panels*, H&E × 200; *lower panels*, CD117 (KIT) immunostaining × 200

studied in the surgical specimens. We investigated exons 9,11,13, and 17 for the *KIT* receptor gene and exons 12 and 18 for the *PDGFRA*, gene, because these exons have been reported as hot spots for mutations. The first surgical specimen had a deletion mutation (codon 564–578 : AAT GGA AAC AAT TAT GTT TAC ATA GAC CCA ACA CAA CTT CCT TAT / 45 bp del) in exon 11 of the *c-kit* receptor. In the specimen from the second operation, a novel point mutation (Asp820His) in the *c-kit* receptor gene, in addition to the deletion mutation, in exon 11, was identified in exon 17 (Fig. 3).

Discussion

GISTs commonly carry an activating mutation of the *KIT* gene [1], or less often, a mutation in the *PDGFRA* gene [5]. KIT is a gene product of the v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT), and shows type III receptor tyrosine kinase activity. Most GISTs have oncogenic *KIT* mutations within exon 9 or exon 11 in the kit receptor gene, which encode parts of the extracellular and intracellular juxtamenbrane regions, respectively. In addition, there are a few GISTs that have mutations within exon 13 or exon 17 in the kinase domain of the kit receptor. All *c-kit* mutations in GISTs, irrespective of the affected domain, appear to be associated with gain of function, as manifested by constitutive KIT tyrosine phosphorylation [1].

Imatinib mesylate (STI 571), a phenylaminopyrimidine derivative, is a small molecule that selectively inhibits the enzymatic activity of several tyrosine kinases, including ABL; the BCR–ABL fusion protein of chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia; PDGFR; and the product of the *KIT* gene [6,7]. Recently, several clinical trials have demonstrated that imatinib was effective for metastatic and recurrent

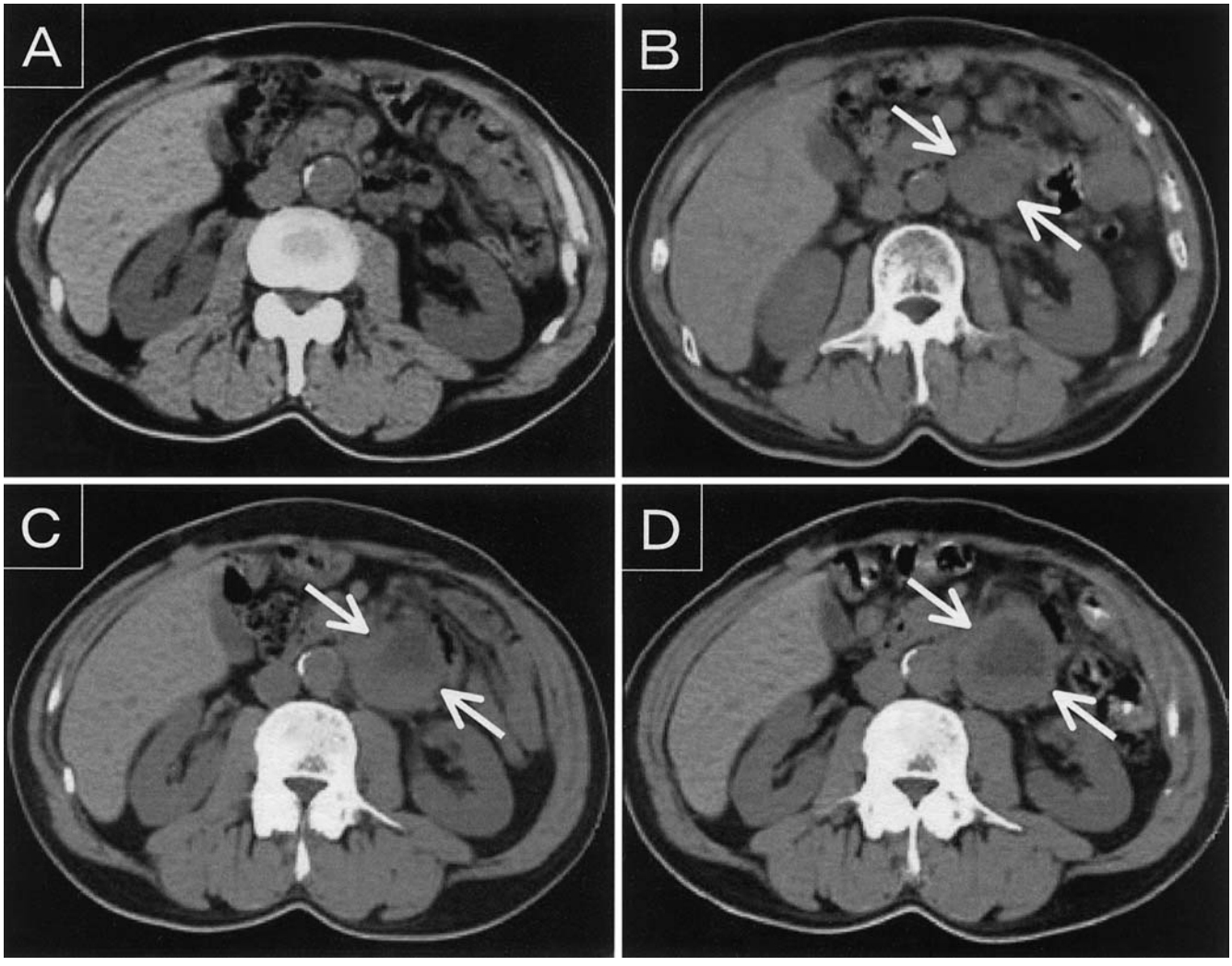

Fig. 2A–D. Serial computed tomography (CT) scans. **A** At 24 months after the first operation, no recurrence was detected. **B** At 30 months, 3 months after the initiation of imatinib dose reduction, a 4-cm tumor was detected (*arrows*). **C** Two months after the time the scan shown in **B** was taken, the tumor size had increased (*arrows*). **D** At 35 months after the first operation, the tumor was 6 cm in diameter (*arrows*). The patient then underwent reoperation

GISTs [8–10]. On the other hand, some GISTs are resistant to imatinib from the beginning (primary resistance), or they acquire resistance after the initial response or disease stabilization (acquired resistance).

The efficiency of and resistance to imatinib treatment for GIST depend on where the mutation is located. In GISTs with the exon 11 mutation in *c-kit*, imatinib treatment is effective. However, the therapeutic effect of imatinib may not be as good if exon 17 is mutated. Exon 17 is located in the tyrosine kinase II domain, and this domain is a portion of the adenosine triphosphate (ATP) binding site. Imatinib competes with ATP for the ATP-binding site of the kinase, preventing downstream signaling. Thus, a point mutation of exon 17 inhibits imatinib binding to the ATP-binding site, resulting in constitutive and strong activation of KIT phosphorylation [2,11].

At present, the mechanism underlying the development of a second mutation in GIST is unclear. It is known that, in patients on prolonged imatinib therapy, resistance is acquired by a median time of 27 months. Several mechanisms for biological imatinib resistance have been suggested [3,12]: (A) acquisition of a new *c-kit* or *PDGFRA* point mutation, coexpressed with pre-imatinib mutations in the same genes, with the resultant strong phosphorylation of KIT or PDGFRA; (B) KIT genomic amplification with overexpression of the KIT oncoprotein, without a new point mutation; and (C) activation of other types of tyrosine kinase receptor.

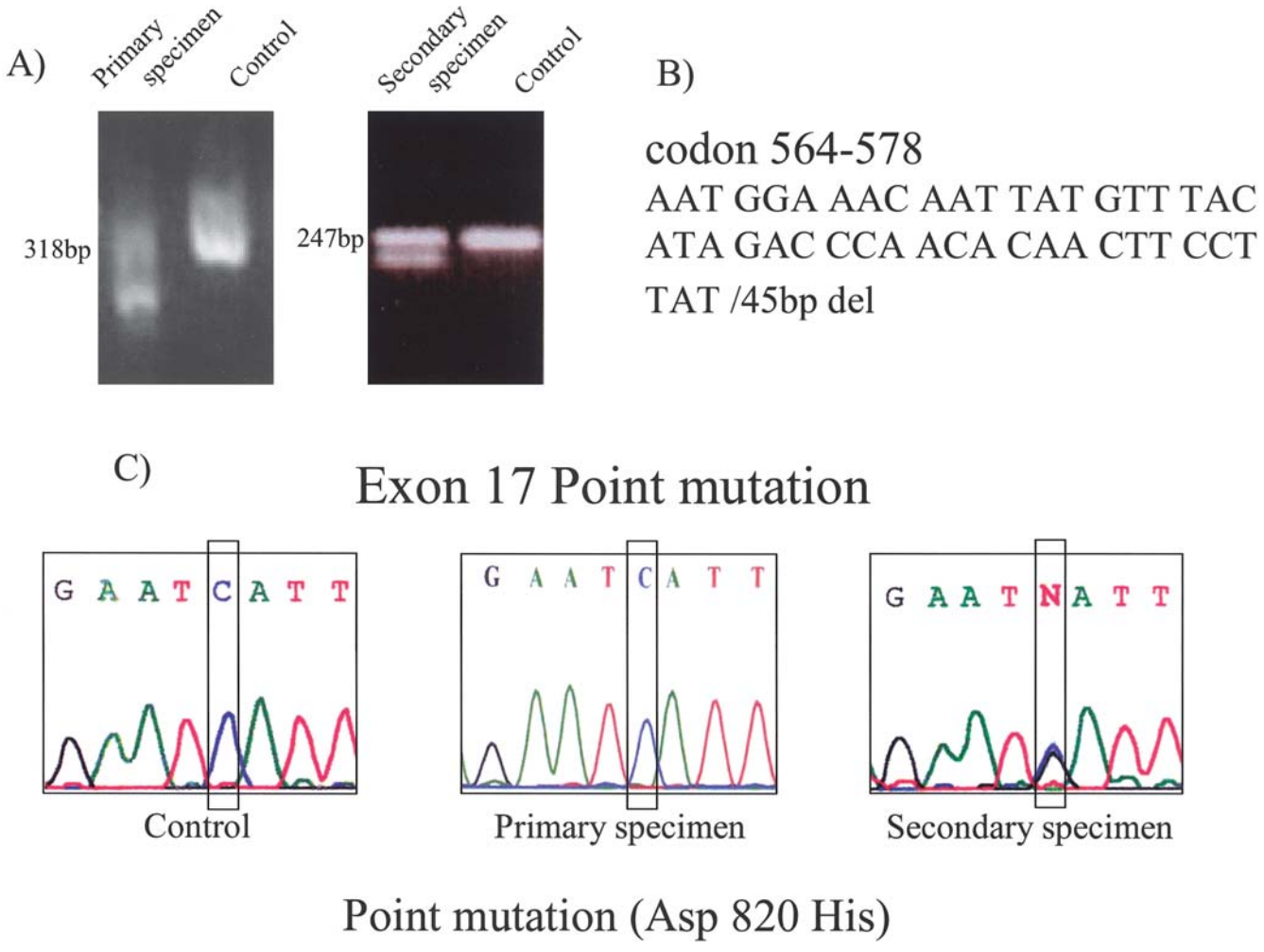

Fig. 3A–C. Analysis of *c-kit* gene DNA from the second surgical specimen. **A** *Left panel* of agarose electrophoresis shows lower band shift of DNA fragment in primary tumor specimen. The same change was observed in DNA from the secondary tumor. DNAs in the *left panel* were extracted from formalin-fixed paraffin embedded tissues, and those in the *right panel* were extracted from frozen specimens. In the primary and secondary specimens, an exon 11 mutation (deletion) in the KIT receptor was identified by sequence analysis of the upper and lower bands. We used human placenta-origin genomic DNA as control. **B** The lower band in the tumor specimens turned out to be a deletion mutation of exon 11 in the *c-kit* receptor gene, shown by the sequence analysis. **C** In the secondary tumor, a novel point mutation (GAT820CAT, Asp820His) in the *c-kit* receptor was identified

In our patient, a novel mutation in exon 17 of the kit receptor was demonstrated in a recurrent lesion after 2 years of treatment, which suggests that the recurrence was caused by mechanism (A) above. Thus, the novel mutation was thought to be related to imatinib resistance, although we are not sure whether the novel mutation was either acquired or selected during the 2 years' imatinib treatment. It has also been documented that the cessation of imatinib or subtherapeutic levels the drug during stable measurable disease results in tumor proliferation [13]. The recurrence and resistance that followed imatinib treatment in our patient may have been related to the dose reduction of imatinib.

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