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Case Report

Successful Treatment with Imatinib Mesylate of a Gastrointestinal Stromal Tumor with *c-kit* exon 11 Mutation

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Gastrointestinal stromal tumors (GISTs) are common form of submucosal tumors of the stomach. Treatment of recurrent GISTs had been unsuccessful because of resistance to chemotherapy and radiotherapy. Recently, GISTs were reported to markedly respond to the molecular target agent, imatinib mesylate. We present here a patient with recurrent GIST and *c-kit* mutation who was successfully treated with imatinib mesylate. A 66-year-old man underwent partial gastrectomy because of GIST. The tumor was 3 cm in size and positive for KIT expression. One year after the excision, spiral computed tomography (CT) scan revealed four intra-peritoneal recurrence lesions measuring 7, 4, 3 and 2 cm in diameter. One week after the CT scan, we started treatment with imatinib mesylate at 400 mg/day, which resulted within two months in 40% decrease in the sum of the longest diameter. The reduction of tumor size continued for more than 6 months. Analysis of the *c-kit* mutation of the primary tumor revealed the deletion of 18 bases in exon 11 (codon 551-557), while other exons showed no mutation. In this report, we showed the effectiveness of imatinib mesylate therapy for the recurrence of GIST, especially with *c-kit* mutation in exon 11.

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Introduction

The gastrointestinal stromal tumor (GIST) is a common mesenchymal tumor of the gastrointestinal (GI) tract.[1,2] and is most commonly found in the stomach (40-70%).[3] Forty to eighty percent of GISTs recur despite histopathologically complete tumor resection.[4] The most common sites of recurrence are the peritoneum and liver, whereas regional lymph node metastases are extremely rare.[3] Metastatic GISTs are often resistant to conventional chemotherapy.[5] Recently, imatinib mesylate (STI-571), which was developed for the treatment of chronic myeloid leukemia (CML), was found to be useful for the treatment of metastatic GISTs, especially those expressing KIT.[6] The position of *c-kit* mutation appears to be related to the effectiveness of imatinib mesylate. Since juxtamembranous (JM) domain mutations of *c-kit* are found in 80% of all GISTs, the therapeutic effect of imatinib mesylate on such GISTs with JM mutations is definitive. In contrast, its effectiveness is not so apparent in GISTs without *c-kit* mutation.[7]

Here we report a case of peritoneal metastasis of GIST with *c-kit* mutation at exon 11 that was successfully treated with imatinib mesylate.

Case report

A 66-year-old man was referred to our department for further investigation of a submucosal tumor (SMT) of the stomach. The SMT was located in the upper part of the stomach and measured 3 cm in size. On October 11, 2001, partial gastrectomy was performed under general anesthesia with open laparotomy converted from laparoscopic surgery because of some difficulties. Macroscopically, 5 mm tumor free margin was observed and serosal injury was not evident; however, microscopic examination revealed some bared tumors at serosa (Figure 1.). We could not identify the cause of serosal destruction. Immunohistochemical examination revealed the tumor was immunopositive for CD34, KIT, and vimentin but negative for

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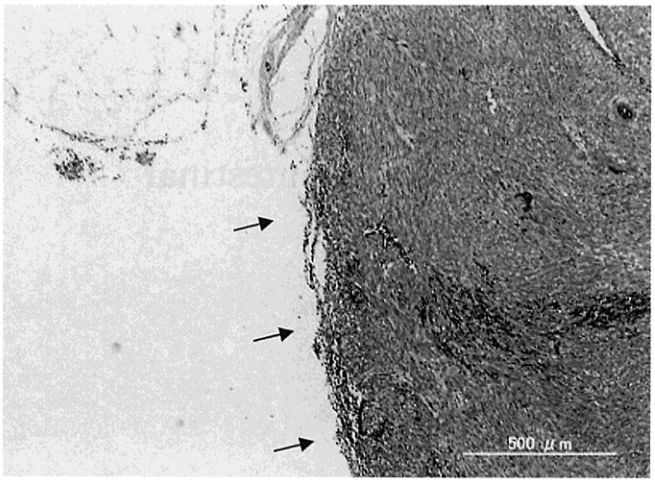

Figure 1. Histological findings of GIST. Spindle cell was abundantly observed. The arrows show the serosal destruction. (H&E ×40)

smooth muscle actin (SMA) and S-100. Examination under high-power field (HPF) showed 18 mitoses per 28. The mass was diagnosed as GIST of uncommitted type, malignant. No recurrence was detected up to one year after the operation, as confirmed by computed tomography (CT) conducted with contiguous 10-mm thick slices. On October 21, 2002, a follow-up CT scan revealed four intra-peritoneal lesions measuring 7, 4, 3 and 2 cm in diameter, although the patient was asymptomatic at that stage and physical examination showed no palpable tumors. The patient consented to receive imatinib mesylate (Glivec, Novartis, Basel, Switzerland) therapy after repeated consultations. One week after the CT scan, we started treatment with imatinib mesylate at a dose of 400 mg/day in the Outpatient Department. Serial CT scans at follow-up showed that the sum of the longest diameter of the peritoneal lesions decreased by 40% and 45% at 2 and 4 months after the initiation of treatment, respectively, and the lack of new lesion (Figure 2). A further regression to 50% and then to 55% at 7 and 11 months, respectively. Thus, the size of the tumor decreased progressively over more than one year after initiation of imatinib mesylate therapy. Edema of the face was observed on the fifth month of treatment, but it diminished following treatment with oral furosemide. No other side effects were observed.

We also analyzed the *c-kit* mutation in the primary tumor as follows. DNA from paraffin-embedded GIST specimen was prepared from five 10-mm thick sections after microdissection, resulting in selection of >90% tumor cells. Genomic DNA was isolated using DXPAT (TaKaRa, Kyoto, Japan) and *c-kit* exons 9, 11, 13 and 17 were amplified by polymerase chain reaction (PCR). The following intron-based PCR primers were designed to amplify exons 9, 11, 13 and 17:

c-kit exon 9: forward-CAAAGTGCTTATTCTTAGACACTTGT,
reverse-AGTGAGTTTGATGACAGTATGGTGT;
c-kit exon11: forward-TGTGCATTATTGTGATGATTCTGAC,
reverse-GAACAAAACAAAGGAAGCCACTG;
c-kit exon13: forward-AGATGCGGCCATGACTGTCGCTG,

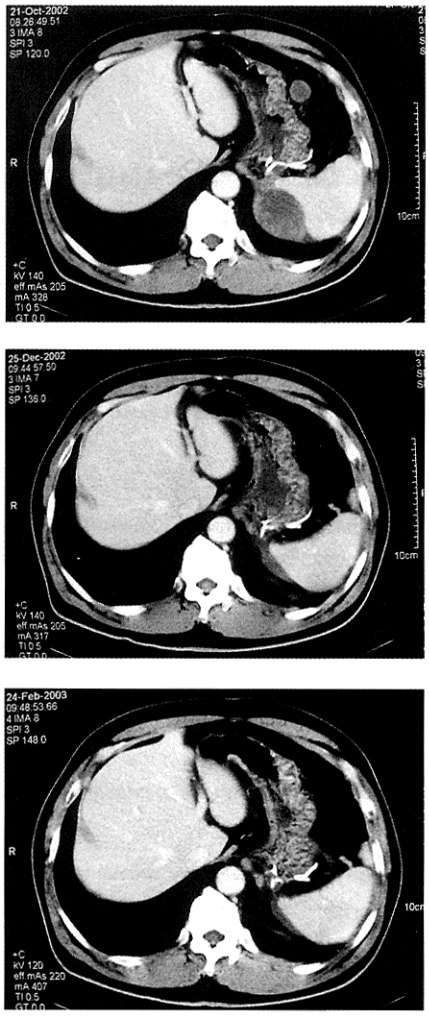

Figure 2. Clinical response to imatinib mesylate therapy. Serial CT scans revealed progressive regression of the peritoneal tumor. Two of the four peritoneal metastases are shown (top). CT scan two months after the initiation of treatment revealed a decrease in tumor size (middle). The tumor size continued to decrease four months after the treatment (bottom).

reverse-CAAGAGAGAACAGTCTGGGTA;
c-kit exon17: forward-ATGTGAACATCATTCAAGGCGTACT,
reverse-ACCATGCAAATTTTGCTGAAGTATAC.

PCR reactions were performed using standard PCR conditions [95°C × 5 min; 94°C × 30 sec, 58°C × 30 sec, 72°C × 30 sec, for 40 cycles; then 70°C × 10 min]. The amplified products were purified by MinElute PCR Purification Kit (Qiagen, Chatsworth, CA) and directly sequenced on an ABI PRISM 3100 automated capillary DNA Sequencer using the BigDye terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). Furthermore, PCR products were subcloned to pGEM-T Easy Vector (Promega, Madison, WI) and sequenced.

Deletion of 18 bases was detected in exon 11 (codon 551-557), while the other exons showed no mutation (Figure 3).

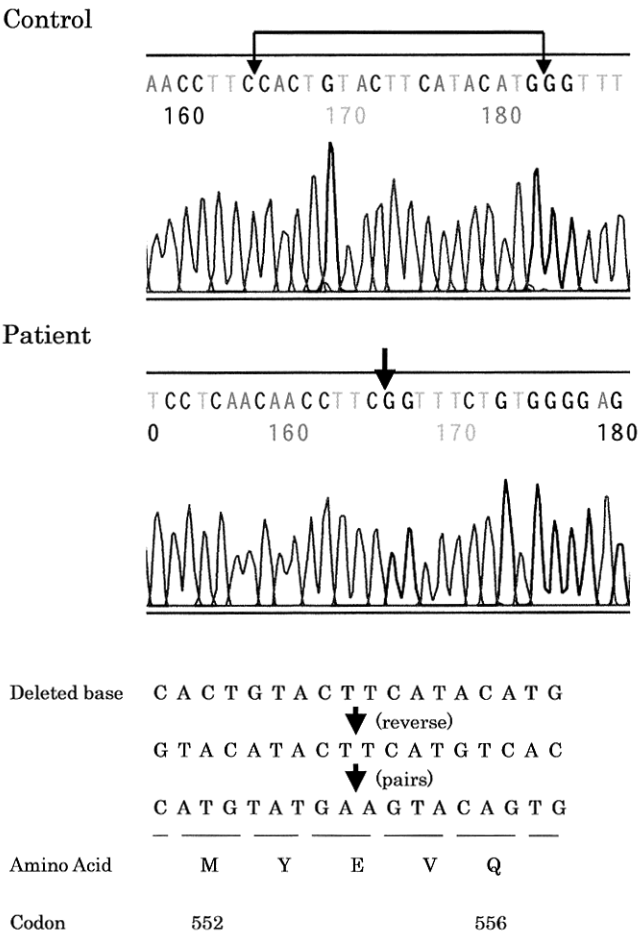

Figure 3. Nucleotide sequences of the patient and control. In the patient, 18 nucleotides, CACTGTACTTCATACATG, were deleted and sequences were changed from CCCATGTATGAAGTACAGTGG (Pro551-Met-Tyr-Glu-Val-Gln-Trp) to CCG (Pro). The results showed deletion of codons extending from Met-552 to Trp-557.

Discussion

We reported here peritoneal recurrence of GIST successfully treated with imatinib mesylate. Peritoneal metastases are most probably the results of tumor cell seeding from the primary tumor directly into the peritoneal cavity. In our case, intra-operative seeding from primary tumor was not evident. The mitotic rate is one of the factors of malignant potential more reliable than those as tumor size. A high mitotic rate of 18 mitosis per 28 HPF was considered one of the causes of recurrence. KIT expression and *c-kit* exon 11 mutation were found in the primary GIST. One year after partial gastrectomy, follow-up CT scan showed peritoneal recurrence. Then we started treatment with imatinib mesylate, a new molecular targeting agent. Two months after the initiation of imatinib mesylate treatment, the tumor size decreased by more than 80% as confirmed by CT scan. Such response continued to be observed for more than 1 year after the initial treatment.

Heinrich et al.[8] reported that patients with exon-11 *c-kit* mutation had a significantly higher response rate to imatinib mesylate therapy (72%) than patients with exon-9 mutation (32%) or without mutation (12%), and the time to treatment failure was also longer in patients with exon-11 mutation. The *c-kit* abnormalities were most frequently observed at codons 557 and 558, and considerable numbers of mutations were also observed in codons 550-556 and 559-562.[9] In our case, subcloning and sequencing revealed the deletion of codons between Met-552 and Trp-557 in exon 11 that encodes the juxtamembrane domain of KIT receptor. In metastatic GIST with exon 11 *c-kit* mutation, imatinib mesylate is the first effective drug, while indication for imatinib mesylate therapy for GIST without exon-11 *c-kit* mutation is controversial. The optimum treatment duration remains unknown, but in metastatic disease, treatment with imatinib mesylate may be needed for at least one year. Furthermore, the optimal treatment for patients with secondary resistance to imatinib mesylate is unknown at present.

KIT expression is not limited to GISTs, other neoplasms may stain positively for KIT in immunohistochemical assays. Reed et al.[10] demonstrated strong cytoplasmic KIT staining in 1.6% of the colon carcinoma patients evaluated. The KIT-positive tumors were poorly differentiated carcinomas arising from the anorectal junction. The remaining tumors revealed no detectable expression of KIT. On the other hand, KIT was expressed in 78% of pancreatic invasive ductal carcinomas.[11] Furthermore, expression of CD117 (KIT receptor) was reported in 95% of Merkel cell carcinomas,[12] in 55% of large cell neuroendocrine carcinoma, and in 28-46% of small cell lung carcinomas.[13,14] In these carcinomas, mesylate imatinib therapy is expected to be positive, and further staining of *c-kit* in other carcinomas especially poorly differentiated carcinomas is also needed. It is expected that carcinomas that overexpress KIT would respond to mesylate imatinib therapy. In addition, analysis of the *c-kit* mutation in these tumors will be helpful for the selection of good responders.

In summary, we presented here a case of GIST with *c-kit* mutation (deletion of 18 bases) in exon 11 (codon 551-557). Peritoneal recurrence of this tumor was successfully treated with imatinib mesylate, a new molecular target agent. Imatinib mesylate might be effective in other carcinomas with *c-kit* mutation, and treatment trial is probably worthwhile.

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