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Case report

Specific mutation in exon 11 of c-*kit* proto-oncogene in a malignant gastrointestinal stromal tumor of the rectum

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Abstract: Gastrointestinal stromal tumor (GIST) in the distal third of the rectum was detected in a 57-year-old man who underwent an abdominoperineal resection of the rectum. Because the tumor expressed CD34 and c-*kit* gene product, but did not express smooth muscle actin or S-100 protein, it was diagnosed as an uncommitted type of GIST. Moreover, a specific mutation in the sequence coding the juxtamembrane domain in exon 11 of the c-*kit* proto-oncogene was revealed by a polymerase chain reaction-single-strand conformation polymorphism method. One year after resection, the patient developed multiple liver metastases. It is suggested that a specific mutation in exon 11 of the c-*kit* proto-oncogene may have played an essential role in the development of the liver metastases.

Key words: c-*kit* proto-oncogene, SSCP-PCR, gastrointestinal stromal tumor (GIST), rectum

Introduction

Gastrointestinal stromal tumors (GISTs) of the rectum are so rare that the determination of malignancy is relatively difficult. The malignancy of stromal tumors has been determined by several factors, such as tumor size and the mitotic activity of the tumor cells.[1] Expression of the c-*kit* gene product and specific mutations in the c-*kit* proto-oncogene have been found in GISTs,[2,3] and the c-*kit* mutations have been reported to occur preferentially in malignant GISTs.[4] We report a patient with malignant rectal GIST who had a specific mutation in the c-*kit* proto-oncogene in the tumor and developed multiple liver metastases 1 year after rectal resection.

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Case report

A 57-year-old man was admitted to the Showa University Fujigaoka Hospital for anal pain and dysuria caused by a rectal tumor. Digital examination revealed a large bleeding tumor fixed at the anterior wall of the rectum. Laboratory data, including examination of tumor markers, showed no abnormality. Rectal endoscopy showed an ulcerated submucosal tumor located 2 cm on the oral side from the anal verge. Abdominal ultrasonogram demonstrated a well marginated heterogeneous tumor compressing the prostate. Magnetic resonance imaging (MRI) revealed a lobulated tumor with central necrosis, exhibiting intra- and extra-mural growth, in the anterior wall of the rectum (Fig. 1). Angiogram of the superior rectal artery showed hypervasculature in the rectal tumor. The preoperative diagnosis was suspected leimyosarcoma of the rectum. On laparotomy, the tumor was palpated under the urinary bladder and an abdominoperineal rectal resection was performed, after checking that there were no metastases to the liver or the lymph nodes.

The resected specimen showed a partly ulcerated submucosal tumor that was 3.5 × 3.5 × 7.0 cm in size. The cut surface of the tumor showed that it was capsulated and solid with central necrosis and bleeding, and exhibited bidirectional growth from the muscularis propria into the mucosa and the adventitia. Microscopically, spindle cells were growing at high density, with cellular atypia and mitosis (Fig. 2). Immunostaining was done on paraffin sections which were deparaffinized and rehydrated without protease exposure. Microwave-based antigen retrieval was used for p53 protein and Ki-67 antigen staining. After blocking with hydrogen peroxide and nonimmune serum was carried out, the sections were incubated with individual primary antibodies, and reactivity was determined with a Histofine SAB-AP(M) kit (Nichirei, Tokyo, Japan). Tumor cells reacted to staining with CD34 (QBEND 10;

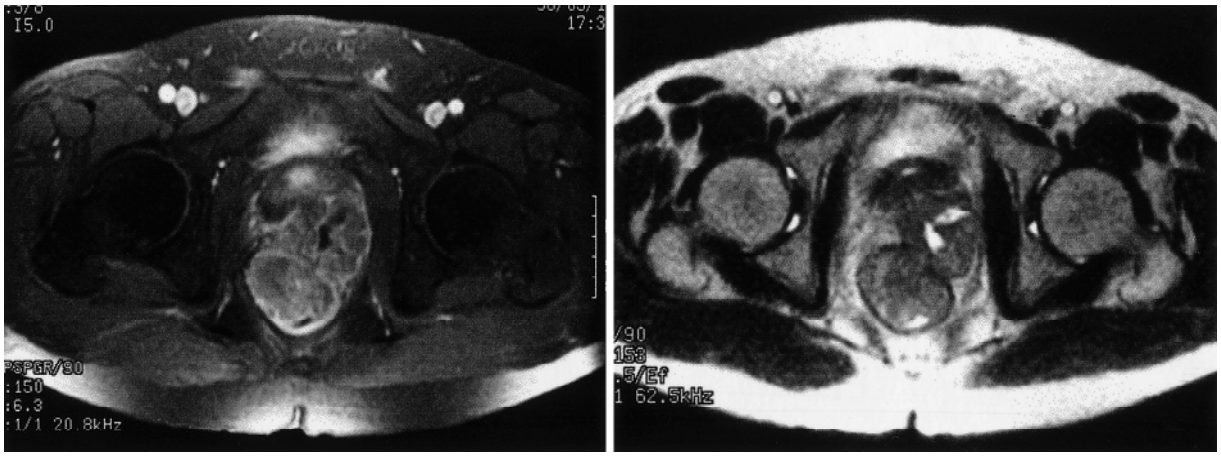

Fig. 1A,B. Magnetic resonance imaging (MRI) of rectal tumor, showing a lobulated tumor with central necrosis, which exhibited intra- and extra-mural growth, in the anterior wall of the rectum. **A** T1-weighted image; **B** T2-weighted image

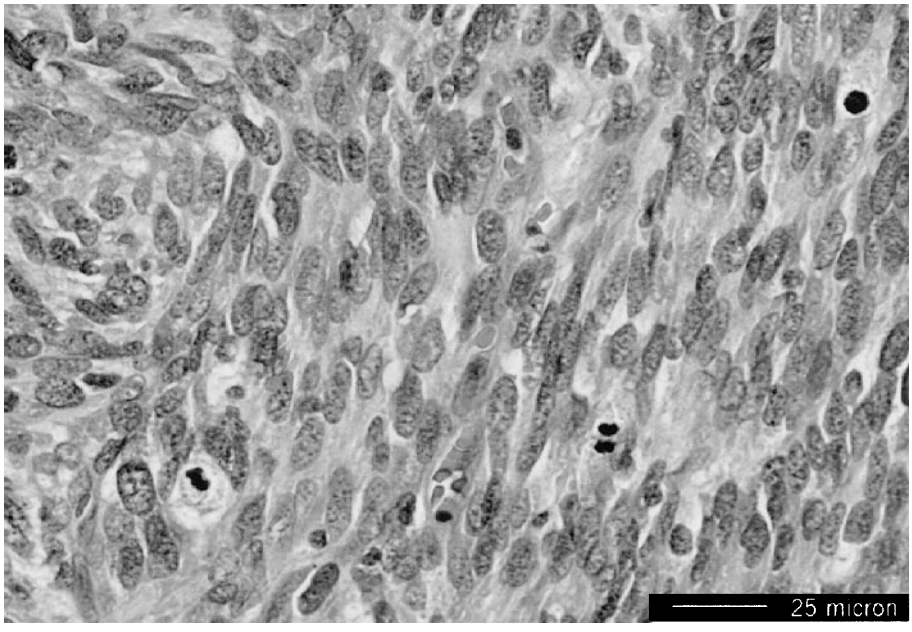

Fig. 2. Microscopic appearance of the tumor, showing that spindle cells were growing at high density, with cellular atypia and mitosis. H&E, ×400

Immunotech, Marseille, France) and c-*kit* gene product (K963; IBL, Fujioka, Japan) (Fig. 3A,B), but did not react to staining with smooth muscle actin (1A4; Dako, Glostrup, Denmark), S-100 protein (Z311; Dako), and neuron-specific enolase (A0587; Dako) (Fig. 4). The percentages of tumor cells which reacted positively to staining with Ki-67 antigen (MIB-1; Immunotech) and p53 protein (CM-1; Novocastra Laboratories, Newcastle, UK) (Fig. 3C,D) were 11.2% and 12%, respectively. Histologically, the tumor was diagnosed as an uncommitted type of GIST[5] in the rectum, with highly malignant potential. DNA samples prepared from the tumor, and the corresponding normal tissue of the muscularis propria, were amplified for single-stranded conformation polymorphism (SSCP) analysis of the c-*kit* and *p53* genes, using polymerase chain reaction (PCR). Gene mutation was recognized in exon 11 of the c-*kit* gene in the tumor (Fig. 5), but not in the *p53* gene (exons 1–11). Sequencing of aberrant single-stranded c-*kit* DNA fragments revealed the deletion of six base pairs, which corresponded to three codons (557–559) for tryptophan, lysine, and valine in the juxtamembranous domain of the c-*kit* gene product (Fig. 6).

The patient's postoperative course was uneventful. He was discharged $1\frac{1}{2}$ months after the operation. One year after the operation, he developed multiple liver metastases (Fig. 7), and was treated with periodic hepatic arterial infusion of cisplatin and 5-fluorouracil.

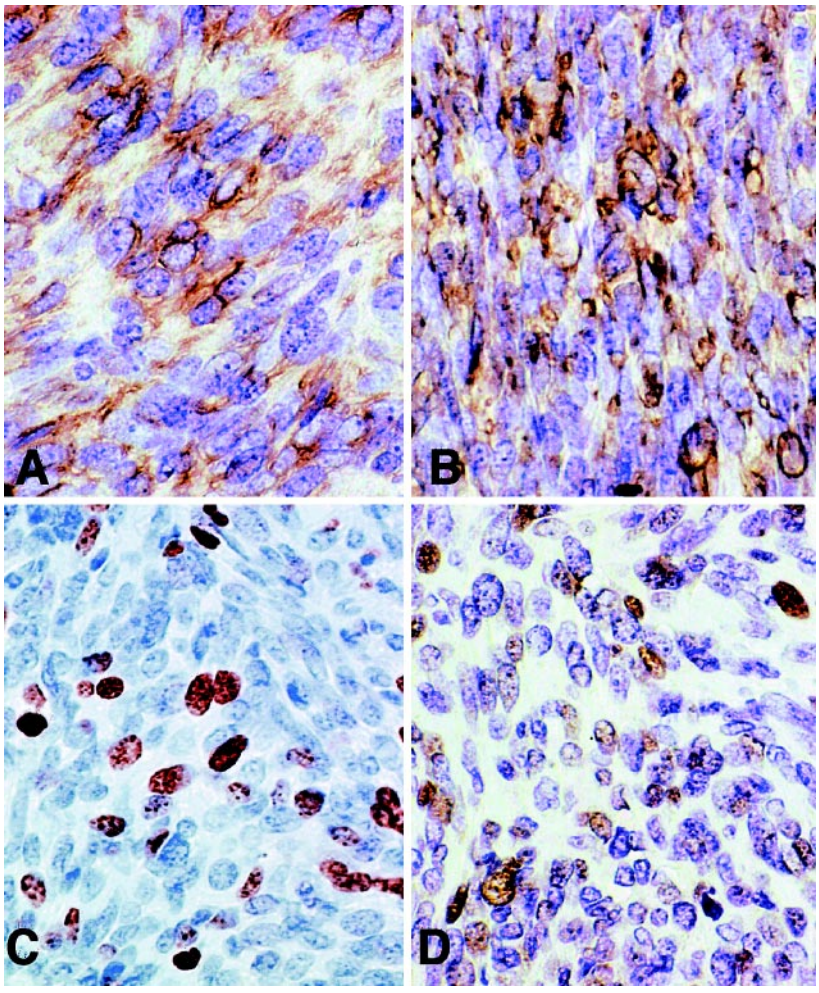

Fig. 3A–D. Microscopic sections demonstrating that tumor cells reacted to antibodies for **A** the c-*kit* gene product; **B** CD34; **C** Ki-67 antigen; and **D** p53 protein. ×400

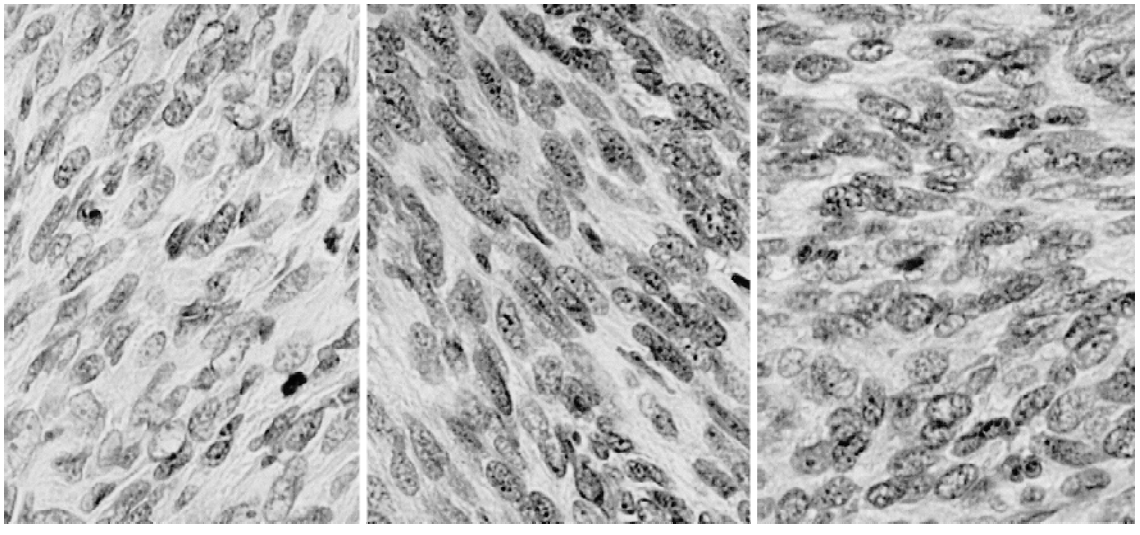

Fig. 4A–C. Microscopic sections demonstrating that tumor cells did not react to antibodies for **A** smooth muscle actin; **B** S-100 protein; and **C** neuron-specific enolase. ×400

Discussion

The malignancy of GISTs has been determined by several factors, such as tumor size, cellularity, cellular atypia, and the mitotic activity of the tumor cells. Of these factors, tumor size and mitotic activity have been reported to be the most reliable for predicting prognosis.[1] However, the average mitotic index and tumor size varied significantly among sites, which were the esophagus, stomach, small bowel, and colon/rectum, and there was a significant difference in site-specific survival.[6] In addition, rectal GISTs in the muscularis propria tend to exhibit malignant behavior, compared with the tumors in the muscularis mucosa.[7]

The present patient exhibited these malignant characteristics, such as location in the muscularis propria, a maximum size of 7.5 cm, high cellularity, cellular atypia, and mitotic activity, which was objectively assessed as 11.2% positive cells with Ki-67 antigen staining.[8] Moreover, the finding of 12% positivity for p53 protein stain-

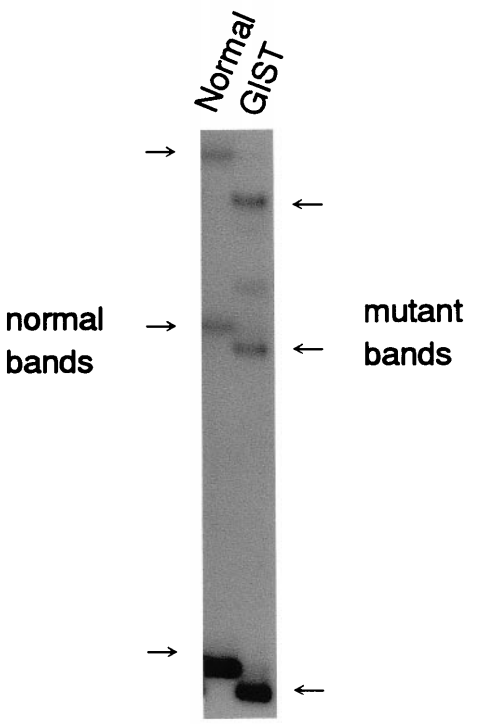

Fig. 5. Single-stranded conformation polymorphism (SSCP) analysis with polymerase chain reaction (PCR), demonstrating a gene mutation in exon 11 of the c-*kit* gene in the rectal gastrointestinal stromal tumor (*GIST*) compared with the corresponding normal tissue of the muscularis propria. Oligonucleotide sequences of the primers for the c-*kit* gene were: (sense) 5′-GTGATCTATTTTTCCCTTTCTC-3′ (4983-5004); (antisense) 5′-CAAAACTCAGCCTGTTT CTGG-3′ (5137-5117)[14]

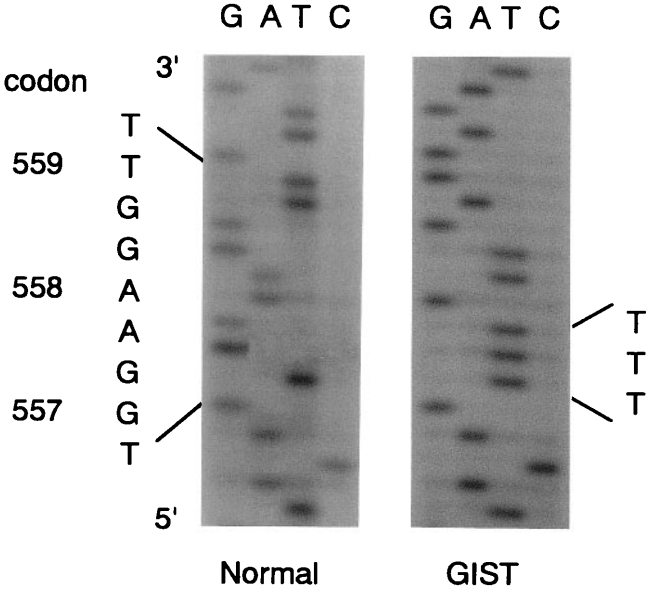

Fig. 6. Specific deletion of six base pairs in exon 11 of the c-*kit* gene in the rectal GIST, detected by sequencing of the aberrant SSCP-PCR DNA fragments, compared with the corresponding normal tissue of the muscularis propria

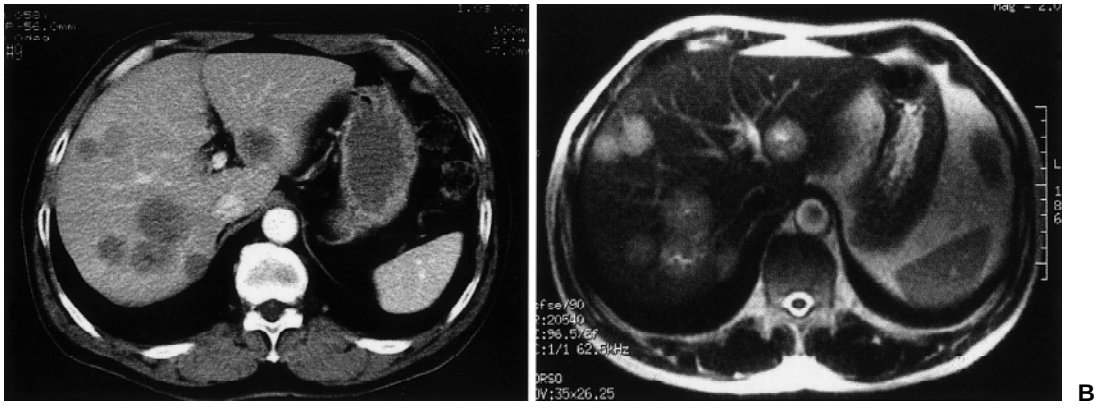

Fig. 7. **A**, Abdominal computed tomography and **B**, MRI showing multiple metastases in the liver 1 year after rectal resection

ing also indicated the malignancy of the tumor,[9] although there were no mutations in exons 1 to 11 of the *p53* tumor suppressor gene. However, it is not known which factors are relevant to the liver metastases of GISTs. Some factors, such as the *nm23* gene,[10] CD44 variant form,[11] and heparanase,[12] have been suggested to play a role in tumor metastasis. It is difficult to determine the molecule or gene responsible for the metastases in GISTs, or in other malignancies, because the metastatic process has been postulated to have multiple steps. The c-*kit* gene may have some roles in the distant metastasis of GIST, although no evidence has so far been reported. Expression of the c-*kit* proto-oncogene has been shown and specific mutations between the transmembrane and tyrosine kinase domains in exon 11 of c-*kit* have been found in GISTs.[2] These mutations have been shown to promote the ligand-independent activation of tyrosine kinase and autonomous cell growth.[2] Recently, c-*kit* mutations have been reported to occur preferentially in malignant GISTs.[4] Mutations in exon 11 were observed in 62% of malignant GISTs which demonstrated clinically malignant behavior by intra-abdominal recurrences or distant metastases. Although other molecular mechanisms[13] have been suggested to explain the differences between benign and malignant GISTs with no mutations in the c-*kit* gene, it is valuable for the prediction of recurrence or prognosis in patients with GISTs to analyze the mutation in the c-*kit* gene of the tumor. The present patient demonstrated a specific deletion in exon 11 of the c-*kit* gene in the tumor and developed multiple liver metastases 1 year after surgery. It is suggested that specific mutations in exon 11 of the c-*kit* proto-oncogene may have played an essential role in the development of the liver metastases.

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