Deletion of the *KIT* gene is associated with liver metastasis and poor prognosis in patients with gastrointestinal stromal tumor in the stomach

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Abstract. The goal of this study was to investigate the association of mutations in the *KIT* gene and the platelet-derived growth factor receptor α (*PDGFRA*) gene with clinicopathological features of patients with gastrointestinal stromal tumor (GIST) localized in the stomach. We evaluated 56 gastric GISTs for *KIT* and *PDGFRA* mutations. DNA was extracted from paraffin-embedded tumor specimens, and exons 9, 11, 13 and 17 of the *KIT* gene and exons 12 and 18 of the *PDGFRA* gene were amplified by polymerase chain reaction and sequenced. The genetic features were then compared with the clinicopathological features. Immunohistochemistry was performed for KIT, CD34, Ki-67 (as a marker of cell proliferation) and CD31 (as a marker of microvessel density), and apoptosis was assessed by *in situ* DNA nick-end labeling. Thirty-four (61%) of the 56 GISTs had a mutation in exon 11 of *KIT*, and 2 (4%) had a mutation in exon 13 of *KIT*. Deletions in exon 11 of *KIT* were the most common mutation encountered in the present study. No mutations were found in exon 9 or 17 of *KIT*. Six of the 20 GISTs lacking *KIT* mutations had a mutation in exon 18 of *PDGFRA*, and 1 had a mutation in exon 12 of *PDGFRA*. The *KIT* mutation-positive GISTs showed more frequent liver metastases and higher mortality than *KIT* mutation-negative GISTs. Our data indicate that *KIT* mutations, especially deletions in exon 11, are markers of poor prognosis for gastric GISTs.

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Introduction

Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of the human gastrointestinal tract. Although stromal tumors can occur throughout the GI tract from the esophagus to the rectum, they occur most frequently in the stomach (1), less frequently in the small intestine, and least often at other sites. GISTs differ from other mesenchymal tumors histologically, immunohistochemically and genetically (2). Only GISTs express KIT, a *KIT* protooncogene protein, and positivity for KIT has been reported in 89-100% of all GISTs (3,4). Sequencing of the *KIT* gene has revealed activating mutations in many GISTs (5). These *KIT* gene mutations have not been observed in other mesenchymal tumors, such as leiomyomas or leiomyosarcomas (2,6). Recently, it has been reported that a subset of GISTs that lacked *KIT* mutations had activating mutations in the related receptor tyrosine kinase, platelet-derived growth factor receptor α (*PDGFRA*) (7).

At present, the best treatment for localized GIST is complete surgical excision; however, relapse is not unusual. Prediction of relapse and metastasis of GISTs on the basis of clinicopathological features is often difficult. In addition, non-operable GISTs, such as metastatic or invasive GISTs, are typically resistant to conventional chemotherapies. Recently, imatinib mesylate (STI571), which was developed for treatment of chronic myeloid leukemia (CML) (8,9), was found to be useful for treatment of advanced GISTs (10-13). Although GISTs are less common than gastrointestinal carcinomas or lymphomas, they have received attention as a model system for development of molecular therapies.

It has been reported that GISTs with *KIT* mutations have poorer prognoses than those lacking these mutations (2,6,14-16). In contrast, there are reports that *KIT* mutations are not associated with malignancy (17,18). Therefore, the prognostic significance of *KIT* mutations remains controversial.

In the present study, we examined the correlation of alterations in exons 9, 11, 13 and 17 of the *KIT* gene and in exons 12 and 18 of the *PDGFRA* gene in gastric GISTs with cell proliferation, angiogenesis and apoptosis. We also

investigated whether alterations in *KIT* are important prognostic markers in gastric GISTs.

Patients and methods

Patients and tumor specimens. We retrospectively analyzed the clinical records of 74 patients who underwent curative surgical resection for mesenchymal tumor of the stomach at Hiroshima University Hospital during the period of 1980-2000. Mesenchymal tumors were identified as GISTs on the basis of positive immunohistochemical staining for KIT and/or CD34. With this criterion, 62 of the 74 tumors (84%) were identified as GISTs. All patients presented with a solitary tumor. Of the 62 patients, complete outcome data were not available for 6 patients, who were therefore excluded from the analyses. None of the remaining 56 patients was treated with STI571 before or after surgery. For strict privacy protection, identifying information for all samples was removed before analysis; the procedure was in accordance with the Japanese Government's Ethical Guidelines for Human Genome/Gene Research.

Immunohistochemistry. Expression of KIT, CD34, Ki-67 and CD31 was analyzed by immunohistochemistry (Fig. 1A-D). A representative tissue block was collected for each case, and immunohistochemistry was performed on 4-μm thick sections of formalin-fixed, paraffin-embedded tissues cut from these blocks using the Envision system (Dako Cytomation, Carpinteria, CA) for KIT and CD34 or the LSAB2 kit (Dako Cytomation) for Ki-67 and CD31.

All tissue sections for immunohistochemistry were deparaffinized in xylene and rehydrated with graded ethanol washes (100-70%). Sections were then treated with pronase (trypsin; Difco, Detroit, MI) for 30 min at room temperature prior to CD34 and CD31 immunostaining and treated by microwave for 15 min prior to Ki-67 immunostaining. Sections were not treated before KIT immunostaining. Endogenous peroxidase activity was blocked with hydrogen peroxide for 10 min at room temperature, and sections were incubated with goat serum for 20 min at room temperature to block non-specific binding. The primary antibodies used were anti-KIT (diluted 1:50; Dako Cytomation, Kyoto, Japan), anti-CD34 (Histofine, Tokyo, Japan), anti-Ki-67 (MIB-1, diluted 1:50; Dako Cytomation, Glostrup, Denmark) and anti-CD31 (Dako Cytomation, Carpinteria, CA). The primary antibody was applied for 30 min at room temperature for KIT, 1 h at room temperature for Ki-67 and overnight at 4˚C for CD34 and CD31. 3,3'-Diaminobenzidine (Merck, Darmstadt, Germany) was used as the chromogen, and sections were counterstained with hematoxylin.

The Ki-67-labeling index (LI) was determined by light microscopy of the area with the greatest number of Ki-67-positive cells. These areas were identified by scanning tumor sections at low power (x40 and x100). The Ki-67 LI was calculated as the percentage of positive cells detected in 1,000 tumor cells.

Microvessel density (MVD) was determined by light microscopy examination of CD31 staining in the area with the greatest number of capillaries and small venules. Areas of high vascularity were identified by scanning tumor sections at low power (x40 and x100). Vessels were counted in the 3 areas of a x400 field (x40 objective and x10 ocular; 0.189 mm^2/field) with the greatest neovascularization, and the average count was noted.

In situ DNA nick-end labeling. DNA strand breaks due to apoptosis were detected *in situ* by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL). This is based on the specific binding of terminal deoxynucleotidyl transferase to 3'-OH ends of DNA. We used this method to detect apoptotic cells in gastric GISTs in 4-μm thick sections of formalin-fixed, paraffin-embedded tissue (ApopTag®; Chemicon, Temecula, CA). In brief, paraffin sections were deparaffinized, rehydrated through a graded alcohol series (100-70%), and washed with distilled water. The tissue was digested with 20 μg/ml proteinase K for 15 min at room temperature. Sections were then treated with 3% H_2O_2 in phosphate-buffered saline (PBS) for 5 min at room temperature and washed with distilled water. After prehybridization treatment, terminal deoxynucleotidyl transferase with digoxigenin-11-deoxyuridine triphosphate (dUTP) and deoxyadenosine triphosphate (dATP) were added to cover the sections, which were incubated in a humidified chamber for 60 min at 37˚C. The sections were then incubated for 30 min at room temperature with an anti-digoxigenin-antibody-peroxidase conjugate before staining with a 3,3'-diaminobenzidine-H_2O_2 solution. The sections were washed thoroughly with PBS between incubation steps and then counterstained with hematoxylin. The percentage of positive apoptotic cells among approximately 1,000 tumor cells was calculated.

Molecular analysis. Genomic DNA was extracted from formalin-fixed, paraffin-embedded GIST tissue. In all cases, exon 9 and exon 11 of the *KIT* gene were evaluated for mutations by polymerase chain reaction (PCR) amplification and direct sequencing of the amplification products. Samples from patients negative for both exon 9 and exon 11 mutations were then screened for exon 13 and exon 17 mutations by the same method. Samples negative for these *KIT* mutations were finally screened for exon 12 and exon 18 of the *PDGFRA* gene.

Exons 9, 11, 13 and 17 of *KIT* and exons 12 and 18 of *PDGFRA* were amplified by PCR with the following primer pairs: *KIT* exon 9, forward TTTGGAAAGCTAGTGGTTCA and reverse ATGGTAGACAGAGCCTAAAC; *KIT* exon 11, forward GATCTATTTTTCCCTTTCTC and reverse AGCC CCTGTTTCATACTGAC; *KIT* exon 13, forward GCTTG ACATCAGTTTGCCAG and reverse AAAGGCAGCTTGG ACACGGCTTTA; *KIT* exon 17, forward TTTCTCCTCCA ACCTAATAG and reverse CCTTTGCAGGACTGTCAAGC; *PDGFRA* exon 12, forward TCCAGTCACTGTGCTGCTTC and reverse GCAAGGGAAAAGGGAGTCTT; and *PDGFRA* exon 18, forward CAGGGGTGATGCTATATCAGC and reverse TAAAGTGTGGGAGGATGAGCC.

PCR product sizes were 238, 174, 193, 180, 260 and 235 bp respectively. PCR conditions were 94˚C for 10 min followed by 50 cycles of 94˚C for 1 min, 57˚C for 1 min, and 72˚C for 1 min and a final cycle of 72˚C for 10 min. PCR products were separated by agarose gel electrophoresis, and visualized by UV transillumination. PCR products were purified with

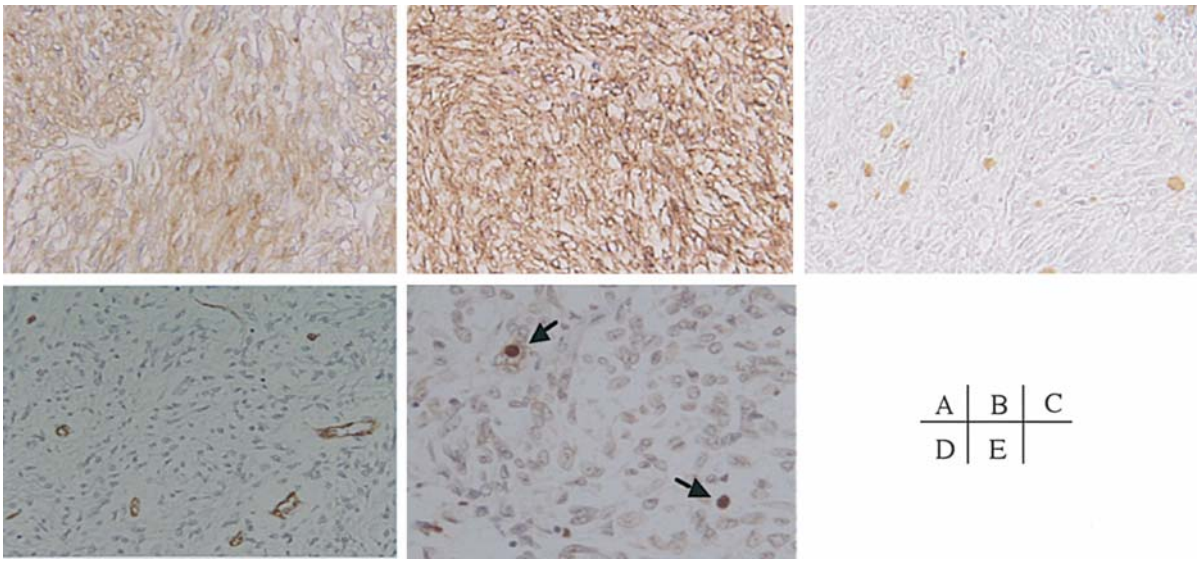

Figure 1. Immunohistochemical assessment of KIT and CD34 expression, proliferation, microvessel density, and apoptosis of gastric GISTs. (A) KIT and (B) CD34, most cells showed strong staining signals. (C) Ki-67 immunostaining. The number of Ki-67-positive cells among approximately 1,000 tumor cells was calculated as a percentage. (D) Immunohistochemical staining of CD31. Microvessels are visible as brown capillaries or small clusters that are distinct from the surrounding tissue. (E) Apoptotic cells stained by TUNEL. Positive nuclei are indicated by arrows.

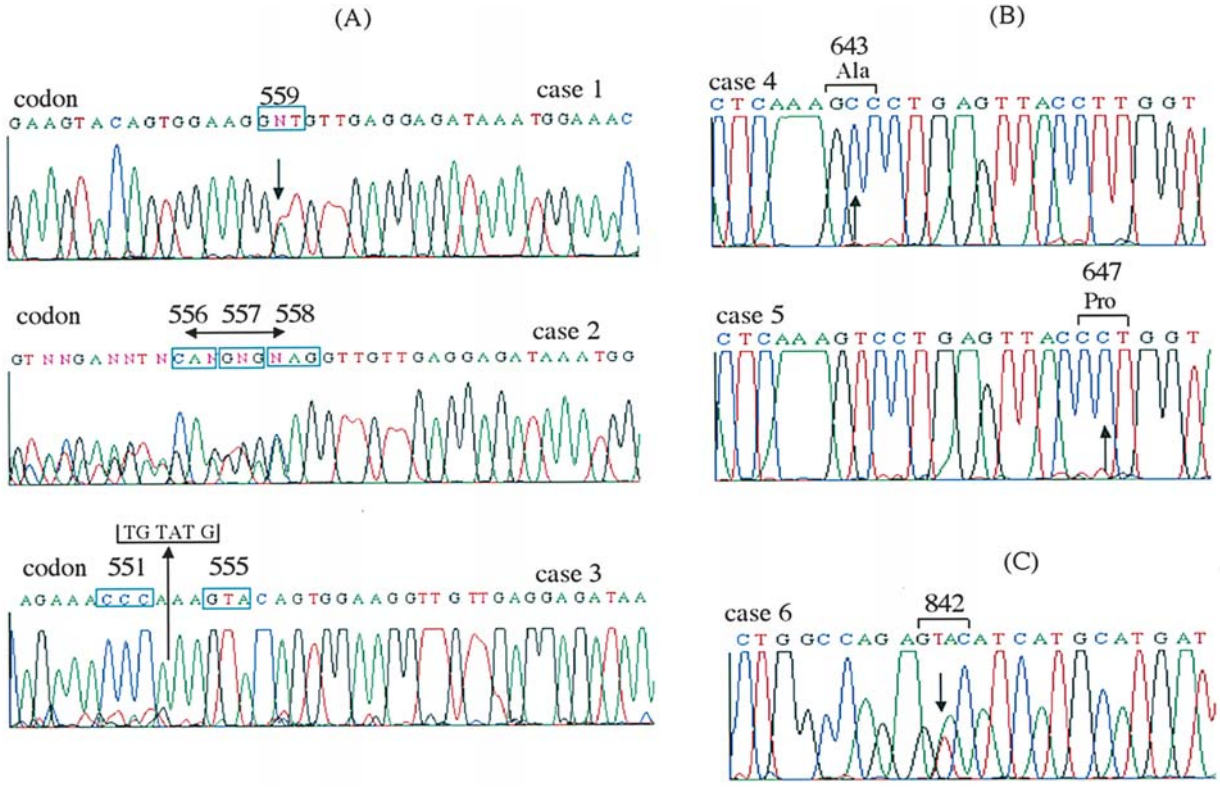

Figure 2. (A) Direct sequencing of mutant *KIT* exon 11 from GIST. All 3 cases shown were sequenced from the antisense direction. Case 1, arrow indicates heterozygous point mutation at the second nucleotide (T→A) of codon 559. Case 2, heterozygous in-frame deletion of 6 bp from codon 556 to 558. Nucleotides (AGTGGA) are deleted at the site indicated by the double-headed arrow. Case 3, homozygous in-frame deletion of 6 bp. Nucleotides (TGTATG) are deleted at the site indicated by the arrow. (B) Direct sequencing of mutant *KIT* exon 13 from GIST. Case 4, arrow indicates a homozygous point mutation of the second nucleotide (T→C) of codon 643, resulting in Val643Ala. Case 5, arrow indicates a homozygous point mutation of the second nucleotide (T→C) of codon 647, resulting in Leu647Pro. (C) Direct sequencing of mutant *PDGFRA* exon 18 from GIST. Case 6, arrow indicates heterozygous point mutation at the second nucleotide (A→T) of codon 842.

the DNA and Gel Band Purification kit (Amersham, Buckinghamshire, UK) before sequencing. *KIT* and *PDGFRA* were sequenced in both the forward and reverse directions with the PCR primers described above. Sequencing was performed with an ABI PRISM 310 DNA Sequencer (Applied Biosystems, Foster City, CA).

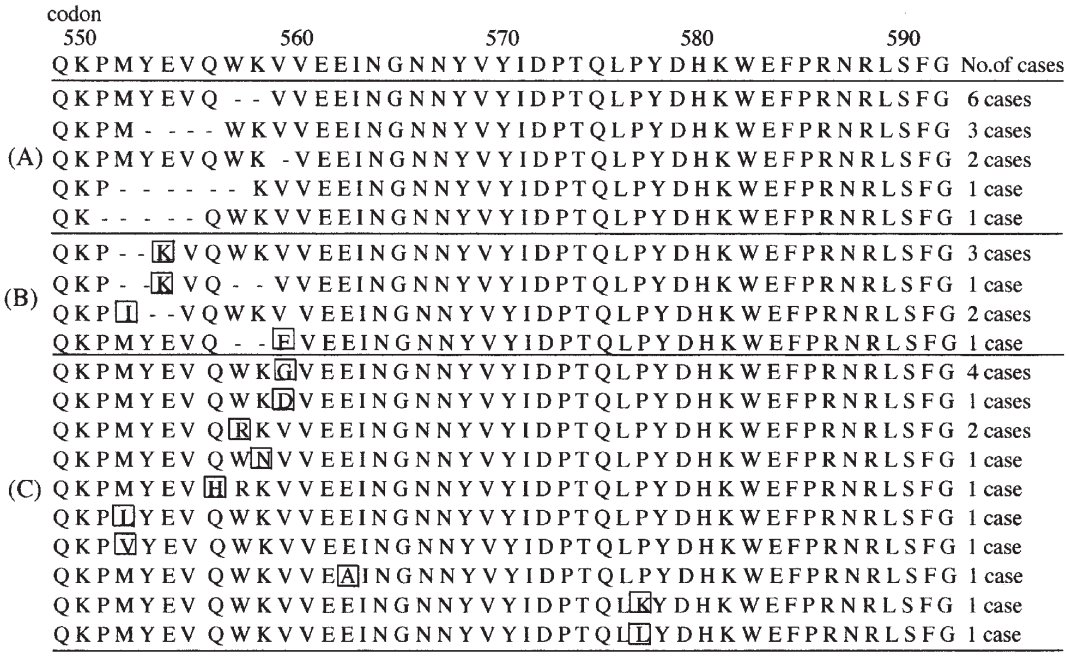

Figure 3. Predicted amino acid sequences of *KIT* with exon 11 mutations. The sequence starts at codon 549 and ends at 592. The wild-type sequence is shown above. Blanks (---) correspond to deletions, and amino acid substitutions are shown in boxes (□). (A) Simple deletions detected in *KIT* exon 11; (B) Deletions and substitutions detected together in *KIT* exon 11; and (C) substitutions alone found in *KIT* exon 11.

Statistical methods. To evaluate the relationships between mutations and clinicopathological factors, data were analyzed by χ^2 or Fisher's exact test in cross tables and by Student's t-test. Survival curves were drawn according to the Kaplan-Meier method and were compared by Fisher's exact test. The significance level was set at 5% for each analysis.

Results

Clinicopathological features. Of 56 selected GISTs, 39 (70%) expressed both KIT and CD34, 12 (21%) expressed only KIT, and 5 (9%) expressed only CD34 (Fig. 1A and B). The patients from whom these GISTs were obtained comprised 35 men and 21 women, and the mean age of the patients at the time of surgical resection was 61 years (range 27-86 years). The mean tumor size was 4.4 cm (range 1-20 cm). During the follow-up period, liver metastases occurred in six patients, all of whom died of the disease within 4 years of surgery.

Evaluation of mutations. *KIT* mutations were detected in 36 (64%) of 56 gastric GISTs. Thirty-four (61%) of these mutations were in exon 11, and 2 (4%) were in exon 13. We did not detect any mutations in exon 9 or exon 17 of *KIT*.

Examples of mutations detected in exon 11 of *KIT* are shown in Fig. 2A. Thirteen cases (23%) showed simple deletions, 14 cases (25%) showed substitutions, and 7 cases (13%) showed substitutions and deletions, not always in the same order (Fig. 3). Deletions were comprised of 3-18 bp, and none disrupted the downstream open reading frame of the gene. In other words, all of the deletions in exon 11 observed in this study were in-frame deletions. The most common mutations in *KIT* exon 11 were a WK (Trp-Lys) deletion at codon 557 in 8 cases and a V559G substitution in 4 cases. In all cases, the deletions contained sequences between codons 550 and 560. Most of the substitutions also occurred in the same region of exon 11; however, a few substitutions were detected in other codons. Thus, most of the mutations in exon 11 of *KIT* were clustered at the 5' end, which is a previously described 'hot spot' (2). Internal tandem duplications (ITDs) at the 3' end of exon 11, which has also been described as a 'hot spot' (18), were not found in the study. In 29 (85%) of the 34 GISTs with exon 11 mutations, we detected *KIT* wild-type, indicating that only 1 of the 2 *KIT* alleles was mutated in these GIST samples. Homozygous mutations were present in 5 (15%) cases.

Two mutations were detected in exon 13 of *KIT*. Both were substitutions; one was Val643Ala and the other was Leu647Pro. The wild-type allele was not detected in either case, and therefore, both were considered to be homozygous (Fig. 2B).

Of 20 GISTs lacking *KIT* mutations, 7 cases (35%) had *PDGFRA* mutations. Six cases were the same substitutions D842V in exon 18 which is the most common *PDGFRA* mutation in GISTs (Fig. 2C) (19), and the other one case was the E563K substitution in exon 12.

Correlation between clinicopathological and genetic features. The relationships between clinicopathological and genetic features of *KIT* are shown in Table I. KIT was expressed in 90% of *KIT* mutation-negative GISTs and in 92% of *KIT* mutation-positive GISTs. CD34 was expressed in 75 and 81%, respectively. Liver metastases occurred in 6 (17%) patients with *KIT* mutation-positive GIST after surgical resection, whereas liver metastasis occurred in none of the patients with *KIT* mutation-negative GIST; however, the difference was not statistically significant. *KIT* mutation-positive GISTs tended to be larger than *KIT* mutation-negative GISTs, and *KIT* mutation-positive GISTs tended to have a large Ki-67 LI (Fig. 1C). MVD assessed by CD31 and apoptotic index

Table I. Correlation between clinicopathological and genetic features of *KIT*.

KIT mutation	Negative (%)	Positive (%)	p-value
Number of cases	20 (36)	36 (64)	
KIT expression	18 (90)	33 (92)	0.834
CD34 expression	15 (75)	29 (81)	0.627
Sex (male/female)	14 /6	21/15	0.388
Age, years (range)	57 (27-75)	62 (35-86)	0.102
Tumor size (cm)	3.7	4.9	0.205
Liver metastasis	0/20 (0)	6/36 (17)	0.053
Ki-67 LI (%)	1.6	2.5	0.155
MVD	14.3	15.2	0.717
Apoptotic index (%)	0.58	0.61	0.810

Ki-67 LI, Ki-67 labelling index; MVD, microvessel density.

Table II. Correlation between clinicopathological features and genetic subtypes of *KIT*.

	Deletions	Substitutions	No *KIT* mutation
Number of cases	20	16	20
Tumor size (cm)	5.4	4.2	3.7
Liver metastasis (%)	5/20 (25)[a]	1/16 (6)	0/20 (0)[a]
Ki-67 LI (%)	2.8	2.2	1.6
MVD	17.6	12.4	14.3
Apoptotic index (%)	0.67	0.47	0.61

Ki-67 LI, Ki-67 labelling index; MVD, microvessel density. [a]$p<0.05$.

assessed by TUNEL in tumors (Fig. 1D and E) showed no relation to *KIT* mutations.

We next classified the *KIT* mutations into 2 subtypes, deletions in the *KIT* gene and substitutions in the *KIT* gene, and we compared these groups and a *KIT* mutation-negative group with tumor size, frequency of liver metastases, Ki-67 LI, MVD and apoptotic index (Table II). A significant difference was detected in the frequency of liver metastases between the deletion mutations and *KIT* mutation-negative groups. A higher frequency of liver metastasis was observed in patients with deletion mutations than in *KIT* mutation-negative patients. With respect to tumor size and Ki-67 LI, these values tended to be largest in the deletion-mutant group, with lower values in the substitution group and the lowest values in the no *KIT* mutation group. However, the differences between these 3 groups were not statistically significant. We did not detect an association between MVD or apoptotic index and any of the groups.

Liver metastasis and death due to the original disease were not observed in the patients with *KIT* exon 13 mutations during the follow-up period. In addition, there were no differences between heterozygous tumors and homozygous tumors with respect to liver metastasis or death due to the original disease (data not shown).

The clinicopathological features of GISTs with *PDGFRA* mutations are shown in Table III. Six patients were male and one was female. Age, tumor size, Ki-67 LI, MVD and apoptotic index were not statistically different compared to those of other groups.

Clinical follow-up. Clinical follow-up was available for all 56 cases, and the median follow-up period for survivors was 56.3 months (range 1-224 months). All 6 patients who experienced liver metastasis during the follow-up period died within 4 years after their original surgery. Kaplan-Meier analysis revealed a strong trend that the survival rate for patients whose tumors expressed a mutant *KIT* isoform was poorer than that for patients with *KIT* mutation-negative tumors (Fig. 4A), although there was no statistically significant association of *KIT* mutations with the rate of death from the original disease (p=0.053).

Overall survival was also analyzed and compared between the 3 groups; *KIT* deletions, *KIT* substitutions and no *KIT*

Table III. Clinicopathological data of GISTs with *PDGFRA* mutations.

No.	Mutation	Sex	Age (years)	Size (cm)	Ki-67 LI (%)	MVD	Apoptotic index (%)
1	D842V	M	60	2.5	1.2	16	0.3
2	D842V	M	59	4.5	1.4	20	0.2
3	D842V	M	65	2.0	1.6	14	2.4
4	D842V	M	63	1.6	0.4	10	0.4
5	D842V	M	62	2.3	4.4	14	1.4
6	D842V	M	57	6.0	1.3	17	0.2
7	E563K	F	66	3.0	0.8	4	0.2

PDGFRA, platelet-derived growth factor receptor α; Ki-67 LI, Ki-67 labelling index; MVD, microvessel density.

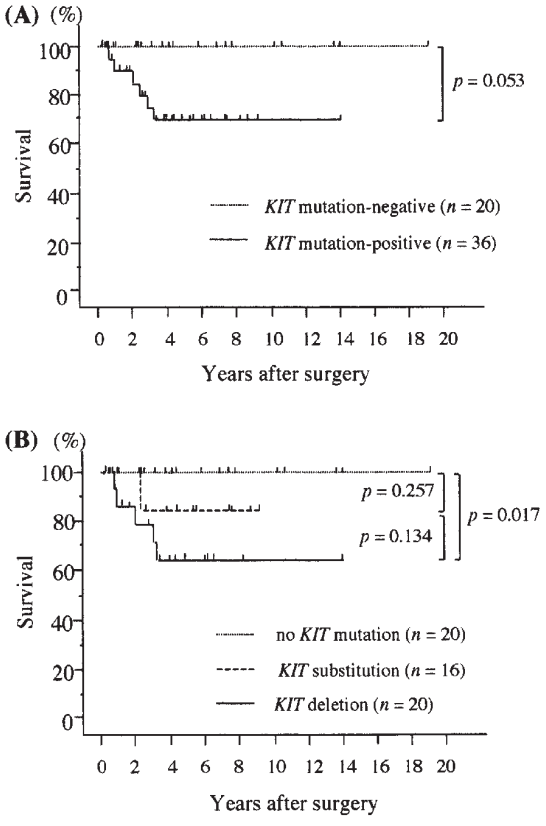

Figure 4. Relation between *KIT* mutations and survival rate. Postoperative survival curves were drawn according to the Kaplan-Meier method. The p-values were calculated with Fisher's exact test. (A) The probability of overall survival for patients with *KIT* mutation-positive and *KIT* mutation-negative tumors. (B) The probability of overall survival for patients with deletion, substitution, and no mutation in the *KIT* gene.

mutations (Fig. 4B). Patients with tumors that harbored *KIT* deletions had a significantly poorer survival rate than those with no *KIT* mutations (p=0.017). There were no statistically significant differences in survival rate between *KIT* deletions and *KIT* substitutions or between *KIT* substitutions and no *KIT* mutations.

Discussion

The *KIT* gene encodes the receptor tyrosine kinase KIT, which is composed of an intracellular tyrosine kinase, a juxtamembrane (JM) region and an extracellular domain with a ligand-binding site. The KIT ligand, stem cell factor (SCF), binds to the KIT receptor, leading to receptor dimerization, activation of kinase activity and autophosphorylation (20). *KIT* mutations that undergo this autophosphorylation without the KIT ligand can contribute to the growth of GISTs (5).

The percentage of GISTs with *KIT* mutations has varied widely in previous studies. The mutations vary from single bp substitutions to complex deletions/insertions but are invariably in-frame mutations. Most have been found in the JM domain, especially between codons 550-560, which is the 'hot-spot' region of exon 11 (2). Taniguchi *et al* (14) reported that exon 11 mutations were present in 71 (57%) of 124 cases, and Lasota *et al* (21) reported exon 11 mutations in 103 (52%) of 200 cases. Other investigators have found exon 11 mutations at much lower rates (<40%) (22,23). In the present study, we detected *KIT* exon 11 mutations in 61% (34/56) of gastric GISTs. There are some possible reasons for the variations in *KIT* mutation rate. One possibility is that the diagnostic criteria for GIST were not consistent across the studies. It is also possible that the locations of GISTs, such as stomach, small intestine, or other sites, were not considered separately. Lastly, the method of DNA extraction from paraffin-embedded tissue or frozen tissue could affect detection of KIT mutations.

The frequency of *KIT* exon 9 mutations appeared to be much lower than that of exon 11 mutations. As previously reported, exon 9 mutations consist of identical tandem repeats of sequences encoding Ala-Tyr, which correspond to codons 502 and 503 (21,24). Most tumors with exon 9 mutations were located in the small intestine. Kim *et al* (15) reported eight cases of GIST with mutations in exon 9, and only two of these cases were localized in the stomach. Hirota *et al* (25) reported seven cases of GIST with mutations in exon 9, and only one case was localized in the stomach. In the present study, we only examined GISTs localized in the stomach, and none of our samples carried a mutation in exon 9 of *KIT*.

In the present study, a mutation in exon 13 of *KIT* was found in 2 cases. Mutations in exon 13 of the *KIT* gene in GISTs are thought to be rare. To our knowledge, there are fewer than 10 reported sporadic GIST cases with mutations in exon 13, and all exhibit the same Lys642Glu substitution (21,24,26). Chen *et al* (27) reported a new coincident mutation in exon 13, Val654Ala, in STI571-resistant GISTs after treatment with STI571. In the present study, we detected two substitutions in exon 13, Val643Ala and Leu647Pro, in GISTs that were not treated with STI571. These substitutions were near the previously reported Lys642Glu site.

It has also been reported that mutations in exon 17 of *KIT* are much more rare in GISTs (14,26). In the present study, no patient carried a mutation in exon 17. The present data suggest that, in gastric GISTs, the *KIT* mutation rates in exons 9, 13 and 17 are very low in comparison to that of exon 11. Therefore, when we evaluate the associations of genetic features of GISTs with clinicopathological features, it is essential to screen for mutations in exon 11 of *KIT*.

We compared clinicopathological features of *KIT* mutation-positive and *KIT* mutation-negative GIST and those of mutation subtypes. Previous studies suggested that *KIT* mutations are more common in malignant than in benign GISTs (2,6,14-16), and our present results support these findings. We compared clinical outcome between *KIT* mutation-positive and *KIT* mutation-negative GISTs and found more frequent relapses of liver metastases and subsequent death due to the disease in patients with *KIT* mutation-positive GISTs. To be exact, 6 of 36 patients with *KIT* mutation-positive gastric GISTs died from the original disease during the follow-up period.

Furthermore, differences between mutation subtypes were detected. Singer *et al* (16) reported that patients with tumors with substitutions in exon 11 had a more favorable relapse-free survival rate than those with other mutations. However, another group found no relation between relapse-free survival rate and *KIT* subtypes (15). In the present study, deletions in exon 11 of *KIT* were associated with more malignant features than substitutions, and patients with such mutations were more likely to experience liver metastasis and die. Therefore,

mutations in the *KIT* gene, especially deletions, may serve as a genotypic marker that correlates with malignancy.

Ki-67 is one of the most reliable prognostic factors for gastric GISTs (28). A high Ki-67 LI is considered an independent predictor of poor prognosis. In the present study, Ki-67 LI tended to be higher in GISTs with *KIT* mutations than in those without *KIT* mutations. Furthermore, when *KIT* mutations were classified into 2 subtypes, deletions and substitutions, the Ki-67 LI was highest in GISTs with deletions, lower in GISTs with substitutions, and lowest in GISTs without any *KIT* mutations. However, these differences were not statistically significant. In contrast, MVD, which is a measure of angiogenesis and is considered a strong indicator of prognosis in patients with a variety of cancers, did not show any relationship with *KIT* mutation-positive or *KIT* mutation-negative GIST or presence or absence of deletions. The growth pathway of GISTs localized in the stomach may differ from that of other gastric cancers, and the mechanism remains to be clarified. We also examined cell apoptosis by TUNEL; we did not observe any differences between *KIT* mutation-positive and *KIT* mutation-negative GISTs.

Recently, it has been reported that a subset of GISTs had mutation in the other site. Heinrich *et al* (7) reported that 35% of GISTs lacking *KIT* mutations had activating mutations in the *PDGFRA* and that mutations in *PDGFRA* and *KIT* were mutually exclusive. In the present study, 7 out of 20 cases of *KIT* wild-type GISTs had mutations in the *PDGFRA* gene.

In conclusion, we have shown that *KIT* mutations, especially deletions which we detected only in exon 11, are associated with occurrence of liver metastasis after complete tumor resection and subsequent death due to the original disease. Post-surgical treatment with STI571 and precise follow-up after surgery may improve the prognosis of patients with a localized gastric GIST with a *KIT* mutation, especially deletion in exon 11.

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