Short Communication

The Complexity of *KIT* Gene Mutations and Chromosome Rearrangements and Their Clinical Correlation in Gastrointestinal Stromal (Pacemaker Cell) Tumors

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Gastrointestinal stromal (pacemaker cell) tumors (GIST/GIPACTs) are frequently associated with activating *KIT* mutations, primarily of exon 11 and rarely of exons 9 and 13, as well as certain chromosome rearrangements. Reports regarding the frequency and prognostic significance of *KIT* mutations are conflicting and few cases have been completely sequenced. Furthermore, there are few detailed analyses of chromosome alterations in GIST/GIPACTs. In a detailed analysis of 14 GIST/GIPACTs from 12 patients, we found a wider spectrum of *KIT* mutations than previously reported, including 11 different in-frame mutations involving exons 11, 14, and 15. No mutations were detected in four malignant tumors. The shorter (GNNK-) *KIT* isoform was preferentially expressed. Cytogenetic and spectral karyotype analyses of 10 tumors revealed clonal abnormalities in eight tumors; the most common were terminal 1p deletions and losses of chromosomes 14 and/or 22. Neither *KIT* mutation status nor chromosome aberrations correlated with tumor phenotype or clinical behavior in our series. Collectively, these findings indicate that the role of *KIT* mutations and chromosomal rearrangements in the pathogenesis of GIST/GIPACTs are more complex than previously recognized. (*Am J Pathol* 2002, 160:15–22)

Gastrointestinal stromal tumor (GIST) is the most common nonepithelial neoplasm of the gastrointestinal tract. Problems regarding criteria for diagnosis, appropriate nomenclature, identification of reliable clinical and mor-

phological prognostic factors, and type of differentiation are well recognized. Recent studies have demonstrated a close phenotypic resemblance between GIST and the interstitial cells of Cajal. Hence, the term gastrointestinal pacemaker cell tumor (GIPACT) has been proposed because it more accurately reflects current knowledge regarding differentiation than the noncommittal and purely descriptive term GIST.^{1,2}

Both interstitial cells of Cajal and GIST/GIPACTs characteristically express the *KIT* protein,³ a type III tyrosine-kinase receptor encoded by the proto-oncogene *KIT*.^{4–6} The ligand for *KIT* is the stem cell factor, also known as Steel factor.⁷ The *KIT* receptor is essential for the development of the interstitial cells of Cajal, which are responsible for initiating and propagating slow wave activity in gastrointestinal muscles as well as mediating motor input from the enteric nervous system.^{8–13}

The occurrence of activating mutations involving exon 11 of *KIT* in sporadic GIST/GIPACTs^{3,14} and the association of exon 11 germline mutations with multicentric GIST/GIPACTs^{15–17} indicate they are important in GIST/GIPACT's tumorigenesis. Reports regarding the frequency of mutations in exon 11, however, vary widely, ranging from 30% to nearly 100%.^{3,18–23} There are also conflicting reports with respect to the association between exon 11 mutations and malignant histology and/or aggressive, malignant behavior. Although some investigators have found a positive correlation between the two and have suggested mutation screening as a potential prognosticator,^{18–21} others have not been able to verify these findings.²² In addition, there have been recent reports of GIST/GIPACTs that lack mutations in exon 11

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Table 1. Summary of Clinical Data, Histology, Mutation Analysis, and Karyotype in 14 GIST/GIPACTs in 12 Patients

Case	Age/ sex	Site	Size (cm)	Histology	Follow-up	Mutation	Karyotype [†]
1	57/F	S.I.	1*	S; B	AW: 8 yrs	PT: V560D, S715del	N.D.
2	70/F	S.I.	7	E + S; Bo	AW: 9 yrs	PT: V560D, S715del	N.D.
3	63/F	G	20	E + S; M	Met: 9, 14 mos TRD: 1 yr	MT (14 mos): W557_K558del	41–45, XX, –21[4][cp4]
4a	72/M	G	19	E + S; M	Met: 1, 2.5, 3.2 yrs	PT: none	44–47, X, –Y[7], +7[2], add(18)(q22-23)[3][cp10]
4b					TRD: 3.5 yrs	MT (3.2 yrs): S715del	41–47, XY, add(18)(q22-23)[2][cp2]
5a	47/M	S.I.	16	E + S; M	Met: 8 mos, 1.3, 2, 2.8, 3, 3.2 yrs	MT (8 mos): V560D, S715del	46, XY
5b					TRD: 4 yrs	MT (3.2 yrs): K558N, K558_V559insP, S715del	41, XY, del(1)(p13), del(2)(p13), der(4)t(4;10)(p16;q22), –7, –10, der(11)t(5;11)(q33;p15), –14, –15, der(18)del(18)(p11)del(18)(q12), der(20)t(7;20)(q11;q13), –22
6	54/F	S.I.	11	S; M	Met: 1.5, 2.2 yrs TRD: 3.7 yrs	MT (2.2 yrs): K558_V560del, K704_N705del, S715del	46, XX
7	78/M	S.I.	4	S; M	Met: APS TRD: 1.5 yr	PT: none	61–68, XY, –X, +1, del(1)(p22), der(1;14)(q10;q10)x2, +2, +3, +4, +5, +6, +7, der(7)inv(7)(q22q32)del(7)(q33)x2, der(7;8)(p10;q10), –8, –9, –9, –10, der(10)t(10;14)(q11;q?), +11, add(11)(p15)x2, add(12)(p13), –13, –14, –14, –15, der(7;15)(q10;q10)x2, +18, add(18)(p11)x2, +19, +20, +20, der(20)t(13;20)(q?;p13), –21, –22, +mar1[cp11]
8	72/F	G	18	E; M	Met: APS TRD: 8 mos	PT: none	44–47, XX, +7[7], –22[3][cp8]
9	66/F	G	33	S; M	Met: 6 mos AWM: 4 yrs	PT: V560D, S715del	41–46, XX, –13[3], –22[5], +mar1[3], +mar2[3][cp7]
10	45/F	S.I.	10	E + S; M	Met: 6 times; 1–9 yrs TRD: 9 yrs	MT (9 yrs): V560D	N.D.
11	50/M	S.I.	18	E + S; M	Met: 2 yrs TRD: 4 yrs	MT (2 yrs): none	42–46, XY, –22[3][cp3]
12	63/M	R	4	S; M	LR: 4 yrs AW: 4.2 yrs	LR (4 yrs): W557_E561del	N.D.

APS, at primary surgery; AW, alive and well; AWM, alive with metastasis; B, benign; Bo, borderline; E, epithelioid; G, gastric; LR, local recurrence; M, malignant; Met, metastases, surgically removed; mos, months; MT, metastatic tumor; PT, primary tumor; R, rectal; S, spindled; S.I., small intestine; TRD, tumor related death, yr/yr: year/years; N.D., not determined.

*Incidental finding at surgery.

[†]Based on G-banding and SKY.

but have activating mutations of exons 9^{24–26} and 13^{24,25,27} of the *KIT* gene.

Alternative splicing in the 3'end of exon 9 of the *KIT* transcript gives rise to two different isoforms of the KIT protein that differ in length by four amino acids and are referred to as GNNK+/GNNK– or KitA+/Kit+.^{28,29} Preferential expression of the shorter isoform of the protein has been observed in a number of neoplasms, including acute myeloid leukemia³⁰ and human germ cell tumors.³¹ Caruana and colleagues³² have shown that the GNNK– isoform is tumorigenic in NIH3T3 cells, whereas the GNNK+ isoform is not. Thus far, there are no reports of the relative distribution of the two KIT isoforms in GIST/GIPACTs.

Previous cytogenetic and molecular cytogenetic studies have shown primarily three recurrent abnormalities,

including terminal deletions of 1p and complete or partial losses of chromosomes 14 and 22.^{33–38}

Our study aimed to investigate the spectrum of *KIT* mutations in GIST/GIPACTs as well as the interrelationship between these mutations, *KIT* isoform expression, cytogenetic and phenotypic characteristics, and clinical behavior.

Materials and Methods

Clinical and Morphological Features

The pertinent clinical and morphological data are summarized in Table 1. Fourteen tumors from 12 patients were analyzed; 6 were primary tumors, 1 a local recur-

Table 2. Summary of Primer Sequences

Name	Sequence
PCR amplification of exons 9–11	
PCRKIT1 s	5' CTATAGATTCTAGTGCATTCAAG 3'
PCRKIT2 as	5' TCAGCCTGTTTCTGGGAAACTCC 3'
PCR amplification of exons 12–15	
PCRKIT9 s	5' CCCAACACAACCTTCCTT 3'
PCRKIT10 as	5' TTGGGACAAACATAAGAAA 3'
Sequencing primers exons 9–11	
SekKIT1 s	5' GTGCATTCAAGCACAAATGGC 3'
SekKIT2 as	5' GAAACTCCCATTGTGATCATAAG 3'
Sequencing primers exons 12–15	
M13F s	5' CAGGAAACAGCTATGAC 3'
M13R as	5' GTAAAACGACGGCCAG 3'
Sp6 s	5' ATTTAGGTGACACTATAG 3'
T7 as	5' TAATACGACTCACTATAGGG 3'
PCR amplification and sequencing of the complete coding region	
PCRKIT13 s	5' TCGCAGCTACCGCGATGAGA 3'
PCRKIT14 as	5' TCACTTCTGGGTCTGTGAGA 3'
PCRKIT15 s	5' CAGACCCAGAAGTGACCAATTA 3'
PCRKIT16 as	5' CTCTCGCTGAAGTATAGTCAAC 3'
PCRKIT17 s	5' GTTGACTATCAGTTCAGCGAGAG 3'
PCRKIT18 as	5' ATTCACGAGCCTGTCGTAA 3'
PCRKIT19 s	5' GCACTTACACATTCCTAGTGTCC 3'
PCRKIT20 as	5' ACATCATGCCAGCTACGAT 3'
PCRKIT21 s	5' ACTCCTTTGCTGATTGGTTTCGT 3'
PCRKIT22 as	5' AATGGTGCAGGCTCCAAGTAGAT 3'
PCRKIT23 s	5' CATGAATATTGTGAATCTACTTG 3'
PCRKIT24 as	5' TGATCCGACCATGAGTAA 3'
PCRKIT25 s	5' GACGAGTTGGCCCTAGAC 3'
PCRKIT26 as	5' AGTTGGAGTAAATATGATTGGTG 3'
PCRKIT27 s	5' TGCTGAAATGTATGACATAATGA 3'
PCRKIT28 as	5' GGTAAGCTACGTTGCTATTG 3'
PCR amplification of <i>KIT</i> isoforms	
PCRKIT3 s	5' GGGGGATCCGATGTGGGCAAGACTTCT 3'
PCRKIT4 as	5' CAGCAAAGGAGTGAACAG 3'

s, Sense; as, antisense.

rence, and 7 metastases (in one patient both the primary tumor and a metastasis were analyzed and in another patient two metachronous metastases were studied). The primary tumors arose in the small intestine ($n = 7$), stomach ($n = 4$), and rectum ($n = 1$) and were histologically classified as predominantly spindled, epithelioid, or mixed-spindled epithelioid as well as benign-appearing, borderline, or malignant based on cellularity, pleomorphism, mitotic activity, necrosis, and growth pattern. Immunohistochemically, all tumors were strongly CD117 (KIT)-positive, 10 of 14 tumors were CD34-positive, and 4 of 14 were focally positive for α -smooth muscle actin. All tumors were negative for desmin, S-100 protein, and chromogranin. Ki67 (MIB-1)-labeling index ranged from <1% in benign and borderline tumors to 5 to 30% in malignant tumors. None of the patients in this study received radiation therapy or chemotherapy; all primary and metastatic tumors were treated solely by surgical resection.

RNA Isolation and Nucleotide Sequence Analyses

RNA was prepared from 14 fresh frozen GIST/GIPACTs using the Fast Prep System (FastRNA Green; Qbiogene, Illkirch Cedex, France). cDNA was prepared with poly

T-primers and RNA as template. All primers (Table 2) were designed using the Unigene Representative Sequence (X06182) for *KIT* mRNA as template.

cDNA from all tumors was amplified in 100- μ l polymerase chain reactions (PCRs) with primers PCRKIT1s and PCRKIT2as designed to amplify exons 9 to 11. In 11 tumors, exons 12 to 15 were also amplified using primers PCRKIT9s and PCRKIT10as. Amplified PCR products were purified with the Jet Sorb gel extraction kit (Genomed, Bad Oeynhausen, Germany). Exons 9 to 11 were directly sequenced, whereas exons 12 to 15 were cloned using pGEM-T-Easy cloning kit (Promega, Madison, WI). Two to eight clones were sequenced from each tumor. Plasmids were prepared using Qiagen Plasmid Mini Kit (Qiagen, Hilden, Germany). All sequence reactions were performed using Big Dye Terminators Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) with the following primers: exons 9 to 11, SekKIT1s and SekKIT2as; exons 12 to 15, M13F and M13R or Sp6 and T7. Sequence reactions were purified by ethanol precipitation and analyzed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems).

In five cases, the complete coding region was amplified and directly sequenced using eight primer pairs (Table 2). Alignments and mutation scanning were performed using Auto Assembler (Applied Biosystems),

BLAST (National Center for Biotechnology Information, Bethesda, MD) and ClustalW (Baylor College of Medicine, Houston, TX). The Unigene Representative Sequence for *KIT* mRNA (X06182) was used for the alignments.

PCR Amplification of the 3' End of Exon 9 of the *KIT* Gene

The expression pattern of the two *KIT* transcript isoforms was analyzed by PCR using cDNA as template. cDNA from human bone marrow and human fetal liver were used as controls. The primers PCRKIT3s and PCRKIT4as were used.³² The PCR products were separated by gel electrophoresis on 4% agarose gel stained with ethidium bromide. Quantification of the PCR products was performed using a Molecular Imager FX (BioRad, Hercules, CA).

Cytogenetic and SKY Analyses

Primary cultures were established from fresh specimens of cases 3, 4a and 4b, 5a and 5b, 6, 7, 8, 9, and 11 as described.³⁹ Chromosome preparations were made from exponentially growing primary cultures, and these were subsequently G-banded and analyzed using standard procedures.⁴⁰

Slides (3- to 5-days-old) were treated with a pepsin solution (12 μ g/ml) for 4 minutes before hybridization. The SkyPaint probe used contained a cocktail of 24 differentially labeled chromosome-specific painting probes (ASI-Applied Spectral Imaging Ltd., Migdal Ha'Emek, Israel). The conditions for hybridization, posthybridization washes, and detection were essentially as recommended by the manufacturer. Chromosomes were counterstained with DAPI (4',6'-diamidino-2'-phenylindole dihydrochloride) containing an anti-fade solution. Image acquisition was achieved with the SpectraCube system (ASI) mounted on a Zeiss Axioplan 2 Imaging microscope equipped with a custom designed optical filter cube (SKY-1; Chroma Technology, Brattleboro, VT) and a DAPI filter.⁴¹ Analysis of spectral images was performed using the SkyView software (ASI).

Results

Mutation Screening

Eleven different *KIT* mutations were detected in 10 of 14 tumors (Figure 1). No mutations were detected in exons 9, 10, 12, and 13. Nine tumors had mutations in exon 11, all between nucleotides 1690 and 1704 (amino acids 557 and 561). Seven of the 11 tumors investigated for mutations in exons 12 to 15 had one or two mutations involving exons 14 and 15, which have not been previously reported in GIST/GIPACTs. These two new mutations included deletion of three nucleotides, 2162 A, 2163 G, and 2164 C, in exon 15, encoding serine in position 715 (7 of 11 tumors) and deletion of six nucleotides, 2129 A,

Figure 1. A: Schematic illustration of the KIT receptor and the location of 11 different mutations involving exons 11, 14, and 15. **B:** Distribution and type of *KIT* mutations in 14 GIST/GIPACTs from 12 patients. WT KIT indicates wild-type KIT amino acid sequence. **Bold**, substitutions; **-**, deletions; **box**, insertion; N.D., not determined; D, aspartic acid; E, glutamic acid; K, lysine; N, asparagine; P, proline; S, serine; V, valine; W, tryptophan.

2130 T, 2131 A, 2132 A, 2133 G, and 2134 A, in exon 14, encoding asparagine and lysine in positions 704 and 705 (one tumor). The mutations were detected in ~50% of the clones from each tumor, indicating that only one allele was mutated. Six of nine tumors with mutations in exon 11 had one or more additional mutations involving exons 14 and 15. All detected mutations were in-frame. No mutations were detected in four tumors in which the complete coding region was sequenced.

GIST/GIPACTs Express Two Isoforms of the *KIT* Transcript

All tumors contained two variants of the *KIT* transcript that differed in length by 12 nucleotides (Figure 2) as shown by PCR amplification and/or sequence analysis. These 12 nucleotides correspond to the following four amino acids: GNNK (position 510 to 513). Thus, GIST/GIPACTs expressed both isoforms of the *KIT* transcript. As shown in Figure 2, there was preferential expression of the shorter isoform with a GNNK-/GNNK+ ratio varying between 1.5 and 2.7.

Cytogenetic and SKY Analyses

Cytogenetic analyses were performed in 10 malignant GIST/GIPACTs from eight patients. Diploid or near-diploid karyotypes predominated in nine tumors; one tumor had karyotypes in the near-triploid mode. The karyo-

Figure 2. PCR amplification of the two different isoforms of the *KIT* transcript illustrating preferential expression of the shorter isoform (GNNK-). Human bone marrow (BM) and fetal liver (FL) with known expression of both isoforms were used as controls. M, 100-bp marker.

Figure 3. **A:** Partial G-banded karyotype of case 5b including, del(1p), del(2p), der(4)t(4;10), der(11)t(5;11), der(18)del(18), der(20)t(7;20), and monosomy 7, 10, 14, 15, and 22. **B:** Spectral and **C:** classified images of a metaphase analyzed of case 5b. **D:** SKY karyotype of the same metaphase as in **B** and **C** showing del(1p), del(2p), der(4)t(4;10), -6, -7, -10, der(11)t(5;11), -14, -15, -17, der(18)del(18), der(20)t(7;20), -21, -22, -22.

types, based on G-banding alone or G-banding in combination with SKY (cases 5b and 7), are shown in Table 1 and Figure 3. Clonal abnormalities were detected in eight tumors. Two tumors had normal karyotypes. Three tumors had simple numerical abnormalities, including; -21; +7 and -22; and -22. Two metastatic lesions (cases 5b and 7) had complex karyotypes with both numerical and structural changes. Detailed comparisons of the G-banded karyotypes with the SKY and DAPI band images allowed clarification of nearly all marker chromosomes in these two tumors. In addition to other abnormalities, both tumors had terminal 1p deletions and losses of chromosomes 14 and 22. Another GIST/GIPACT (case 8) contained a variant cell, which in addition to -22, had a del(1)(p32).

Clinical Correlation

Two different mutations involving exon 11 (substitution) and exon 15 (deletion) were detected in the only benign GIST/GIPACT in our series (<1 cm in size and an incidental finding in the small intestine). Two different mutations involving exon 11 (substitution) and exon 15 (deletion) were also found in a 7-cm borderline tumor of the small intestine (the patient is alive and well 9 years after surgery). Of 10 patients with histologically malignant tu-

mors, 9 developed metastases, 1 had a local recurrence, and 8 died within an average of 2 years (range, 8 months to 4 years). Mutations were detected in 8 of 12 tumors from these patients, including 1 of 4 primary tumors, 6 of 7 metastases, and 1 local recurrence. The mutations consisted of 19 deletions (exons 11, 14, and 15), six substitutions (exon 11), and one insertion (exon 11).

Multiple tumors were analyzed in two cases (Table 1, cases 4 and 5). The primary tumor in case 4 revealed no mutations, whereas the metastasis had one mutation involving exon 15. Both the primary tumor and the metastasis in this case had similar chromosome rearrangements. Two metastases occurring 8 months and 3 years after the primary tumor were analyzed in case 5. The first metastasis had two mutations involving exons 11 and 15 whereas the later metastasis had two different mutations in exon 11 as well as the same deletion in exon 15 as the first metastasis. The first metastasis had a normal karyotype and the later metastasis had a hypodiploid karyotype with complex rearrangements including del(1p), -14, and -22 (Figure 3).

Discussion

Our study indicates that *KIT* mutations in GIST/GIPACTs and their correlation with phenotype, chromosome abnor-

malities, and clinical behavior are more complex than previously recognized. Activating mutations within the 5'-end of exon 11 clustered around nucleotides 1669 to 1701 (amino acids 550 to 561) were most common in our series as in other reports.^{3,15,19,21,22,42} We did not detect mutations in exon 9, 1530ins6, (A502 and Y503) or 13, 1945 A → G (E642K) as reported by others.²⁴ These findings correspond with two large studies of 200 and 133 GIST/GIPACTs in which exon 9 mutations were found to be rare (3% and 5%, respectively)^{25,26} and exon 13 mutations even rarer (1%).²⁵

In addition to reported mutations in exon 11, we also found two new mutations involving exons 14 and 15 in benign, borderline, and malignant tumors and detected multiple mutations in different exons. Although not previously described in the literature, multiple mutations were quite common in our series; six of nine tumors with mutations in exon 11 had one or two additional mutations in exons 14 and 15. The functional consequences of these additional mutations that occur in the intracellular domain of the KIT receptor are unclear and require further exploration in those tumors that also have activating mutations of exon 11. Signaling events, such as binding of phosphatidylinositol 3-kinase (PI3K) and activation of the mitogen-activated protein kinase pathway, need further exploration.^{43–45}

All mutations in our series resulted in in-frame transcripts. Constitutive autophosphorylation without stem cell factor stimulation has also been reported in transfection studies of GIST/GIPACTs with mutations of exons 9, 11, and 13.^{3,14,26,27} These observations suggest that the broad spectrum of *KIT* mutations in GIST/GIPACTs does not reflect general genetic instability and that the tumor cells select for mutated proteins.

The preferential expression of the shorter isoform (GNNK-) in GIST/GIPACTs has not been previously reported and is similar to expression patterns reported in other malignancies such as acute myeloid leukemia³⁰ and germ cell tumors.³¹ The shorter isoform has also been shown to be tumorigenic in NIH3T3 cells, whereas the GNNK+ isoform has not.³² The two isoforms arise because of alternate 5' splice donor sites in the 3'-end of exon 9. The 7 nucleotide-long tandem repeat (AAAG-GTA) occurring in the 3'-end may account for the alternative splicing. The isoform expression pattern of normal interstitial cells of Cajal has not been analyzed, hence the biological significance of preferential GNNK-expression in GIST/GIPACTs is unknown. The shorter isoform has been shown to display stronger receptor phosphorylation and activation of the mitogen-activated protein kinase pathway than the GNNK+ isoform.³² Preferential expression of the shorter isoform could be significant in tumors lacking activating mutations and serve as an alternative mechanism for increased KIT signaling.

Cytogenetic and SKY analyses of 10 GIST/GIPACTs revealed several recurrent structural and numerical abnormalities, including terminal deletions of 1p in two cases (another case had a single variant cell with a 1p deletion), gain of chromosome 7 in three cases, and losses of chromosomes 14 and 22 in two and five cases, respectively. Two cases had 1p deletions, -14, and -22.

These three abnormalities are the most commonly reported clonal changes^{33–38} identified by fluorescence *in situ* hybridization, comparative genomic hybridization, and loss of heterozygosity analyses.^{23,46–50}

To date, no recurrent translocations have been identified in GIST/GIPACTs. Bardi and colleagues³⁴ have described a case with a derivative chromosome 20 resulting from a t(13;20)(q12;p13). A similar or identical translocation was observed in our case 7 that was near-triploid and showed losses of chromosomes 14 and 22. Most of the abnormalities detected in our series of GIST/GIPACTs consisted of losses of whole or parts of chromosomes, suggesting that loss of multiple chromosomal regions, presumably containing putative tumor suppressor genes, might be an important genetic event in GIST/GIPACTs.

The problem of predicting clinical behavior based solely on morphological features in GIST/GIPACTs is well known. The potential utility of detecting activating *KIT* mutations and/or chromosome abnormalities as markers of aggressive behavior has therefore aroused great interest. There are a number of recent studies attempting to correlate mutations and/or chromosome abnormalities with histological features and clinical course. The results of these studies have been conflicting; although some have shown a clear correlation between mutations, histology, and clinical behavior,^{18–21,46,47,49} others have not.²² These studies have focused on mutations in exon 11 and are thus quite limited considering the broad spectrum of mutations occurring in GIST/GIPACTs.

Although our series is relatively small compared to other correlative studies, the mutations occurring in primary, recurrent, and metastatic tumors have been extensively analyzed. The two histologically benign and borderline tumors in our series with long-term disease-free survival had mutations in exons 11 and 15. In contrast, 4 of 12 histologically and clinically malignant tumors in which the entire coding region was sequenced had no mutations. These findings indicate there is no clear correlation between activating *KIT* mutations and biological behavior.

The complexity of GIST/GIPACT mutations and chromosome rearrangements is further demonstrated by two patients with metastatic tumors in our series. In case 4 (Table 1), the metastases had a mutation in exon 15 whereas the primary tumor had none. In case 5, the first metastasis had two mutations (exon 11 V560D and exon 15 S715del) whereas a later metastasis had three mutations. Two of three mutations differed from the first metastatic tumor and involved exon 11 (K558N, K558.V559insP) as well as exon 15 (S715del). These findings suggest that the tumor cell population of GIST/GIPACTs is heterogeneous.

In summary, the observations of clinically benign GIST/GIPACTs with multiple *KIT* mutations involving different exons, metastasizing tumors lacking mutations, as well as the loss and addition of mutations during the course of the disease, all indicate that the correlation between *KIT* mutations and clinical behavior is far more complex than initially appreciated. Apparently a malignant phenotype may be acquired without activating mutations and multiple mutations may occur in clinically benign tumors. De-

tection of multiple and variable *KIT* mutations in primary tumors, metastases, and different generations of metastases in the same patient suggest the existence of subpopulations of tumor cells with different mutations.

Our findings are particularly relevant with regard to a recent report in which a patient with metastatic GIST/GIPACT was successfully treated with the newly developed phenylaminopyrimidine derivative, STI571 (Glivec; Novartis, Basel, Switzerland).⁵¹ This drug selectively inhibits the tyrosine signaling of a group of closely related tyrosine kinase receptors, including KIT, PDGFR, and ABL.⁵² In addition to large scale drug trials in leukemia, there are ongoing trials evaluating its efficacy in the treatment of unresectable or metastatic GIST/GIPACTs. Thus, continued investigation of the wide, complex spectrum of *KIT* mutations as well as their functional consequences in a large series of GIST/GIPACTs correlated with phenotype, clinical behavior, and treatment response is imperative. Further studies analyzing whether treatment effects are restricted to GIST/GIPACTs with KIT receptor signaling alterations secondary to activating mutations are also needed.

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