[CANCER RESEARCH 61, 8118–8121, November 15, 2001]

Advances in Brief

KIT Activation Is a Ubiquitous Feature of Gastrointestinal Stromal Tumors

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Abstract

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract, and they are generally resistant to chemotherapy and radiation therapy. Most GISTs express the KIT receptor tyrosine kinase protein, and a subset of GISTs contain activating mutations within the KIT juxtamembrane region. We evaluated 48 GISTs, including 10 benign, 10 borderline, and 28 malignant cases, to determine whether KIT expression and activation are general properties of these tumors. Immunohistochemical KIT expression was demonstrated in each case. Somatic *KIT* mutations were found in 44 tumors (92%), of which 34 (71%) had juxtamembrane region mutations. Other GISTs had *KIT* mutations in the extracellular region ($n = 6$) and in two different regions in the tyrosine kinase domain ($n = 4$). Contrary to previous reports, *KIT* mutations were not identified preferentially in higher-grade tumors: indeed, they were found in each of 10 histologically benign GISTs. Notably, mutations in all KIT domains were associated with high-level KIT activation/phosphorylation, and KIT activation was also demonstrated in the four GISTs that lacked detectable *KIT* genomic and cDNA mutations. These studies underscore the role of KIT activation in GIST pathogenesis, and they suggest that activated KIT might represent a universal therapeutic target in GISTs.

Introduction

GISTs[3] are the most common mesenchymal tumors of the gastrointestinal tract. GISTs are heterogeneous histologically, being most often composed of spindle cells but sometimes having epithelioid features. GISTs were most often classified, until recently, as leiomyomas and leiomyosarcomas, but are now known to represent a discrete neoplastic entity, possibly arising from a progenitor related to the ICC (1–4). The ICC are a network of innervated cells that coordinate peristaltic activity throughout the gastrointestinal system, and there is evidence that the ICC stem cell can differentiate toward either a smooth muscle or ICC phenotype (5, 6). Expression of nonconstitutively activated KIT receptor tyrosine kinase plays a critical role in ICC differentiation and proliferation, both embryologically and postnatally, and KIT expression is characteristic of most true GISTs but not of gastrointestinal smooth muscle tumors and other KIT-negative stromal tumors, which are sometimes grouped with GISTs (5–7). Recently, a subset of GISTs were shown to have oncogenic mutations involving *KIT* exon 11 (8–12). *KIT* exon 11 encodes the KIT receptor juxtamembrane domain, which is the cytoplasmic portion of the receptor immediately following the transmembrane domain. The KIT juxtamembrane domain is pivotal in KIT signal transduction, both through interactions with various adapter proteins and phosphatases and through modulation of KIT catalytic activity. GISTs with *KIT* exon 11 mutations are reported to be a higher grade, or associated with poorer outcome, than those that lack such mutations (9, 11, 12).

KIT is a type III receptor tyrosine kinase that is activated when bound by a ligand known as steel factor or stem cell factor (13). KIT is essential to the development of hematopoietic progenitors, mast cells, germ cells, melanocytes, and the ICC (7, 14–16). Notably, oncogenic *KIT* mutations have been implicated in neoplasms arising from these cell lineages, including mast cell tumors, myelofibrosis, chronic myelogenous leukemia, germ cell tumors, and GISTs (8, 17–23). Interestingly, the locations of these mutations are nonrandom, and they vary according to tumor type. For example, KIT mutations in myelofibrosis and chronic myelogenous leukemia involve the KIT receptor extracellular region, whereas mutations in mast cell tumors and germ cell tumors involve predominantly the intracellular kinase domain. These observations suggest that the preferential mechanisms of KIT oncogenic transformation might vary in different tumor types. There are several potential explanations for histology-associated mutation clustering. One likely possibility is that expression of the many known KIT-interacting proteins, including those that inhibit KIT signaling, vary depending on the cell lineage and differentiation. Hence, a KIT mutation with oncogenic activity in one cell type might lack such activity in another.

The hypothesis in the present study was that KIT, in light of its essential role in ICC differentiation/proliferation, might have a central oncogenic function in most GISTs. We addressed this hypothesis by evaluating mutations throughout the *KIT* coding sequence in a series of 48 GISTs from a single institution. We also determined whether mutations in various KIT domains were associated with constitutive receptor activation (phosphorylation). Finally, we performed clinicopathological correlations to evaluate whether KIT mutations might be associated with differences in histology and predicted clinical behavior.

Materials and Methods

Patients. Patients were accrued at Brigham and Women's Hospital from the years of 1990–2000. Study inclusion criteria were a histologically confirmed diagnosis of GIST, determined either prospectively or retrospectively, and availability of tissue snap-frozen from the tumors at the time of surgical resection.

The patients included 27 men and 21 women at a median age of 56 years (range, 24–84 years). The histological features of all 48 tumors were evaluated by two of the authors (B. P. R. and C. D. M. F.), and a minimum of one 4-μm-thick H&E section was examined per centimeter of tumor diameter. Histological grade was assigned by following exactly the published criteria of Newman *et al.* (24), in which grade is based on mitoses per 30 HPF, spindle cell *versus* epithelioid histology, and presence of atypia or pleomorphism. Criteria for benign tumors included a spindle cell lesion without atypia and with two or fewer mitoses per 30 HPF, or an epithelioid lesion with no mitoses

Received 7/24/01; accepted 10/2/01.

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[3] The abbreviations used are: GIST, gastrointestinal stromal tumor; ICC, interstitial cells of Cajal; HPF, high-power field.

per 30 HPF. Criteria for malignancy included a spindle cell lesion without atypia but with more than five mitoses per 30 HPF, or a spindle cell lesion with frank atypia and with three to five mitoses per 30 HPF, or an epithelioid lesion with more than two mitoses per HPF. Lesions with parameters intermediate between those for benign or malignant were classified as borderline. Using these criteria, the study tumors included benign ($n = 10$), borderline ($n = 10$), and malignant ($n = 28$) cases. The median tumor size was 10 cm in maximal diameter (range, 2–30 cm). Tumor cell morphology was spindle cell ($n = 32$), mixed spindle cell and epithelioid ($n = 13$), or pure epithelioid ($n = 3$). Primary sites were gastric ($n = 28$), small bowel ($n = 16$), and peritoneal dissemination at presentation ($n = 4$). Immunohistochemical analyses were performed using the avidin-biotin-peroxidase complex method. The following antibodies were used: KIT (A-4052, polyclonal, 1:100 dilution; DAKO Corp., Carpinteria, CA), CD34 (QBEND 10, monoclonal; 1:10 dilution: Serotec, Washington, DC), SMA (1A4, monoclonal, 1:5000 dilution; Sigma Chemical Co., St. Louis, MO), S-100 protein (polyclonal, 1:600 dilution; DAKO), and Desmin (D33, monoclonal, 1:150 dilution; DAKO).

KIT Sequence Analyses. Initial mutation screening was performed by genomic sequencing of exon 10, intron 10, and exon 11. Cases lacking exon 11 mutations were then evaluated further by cDNA sequencing of exons 1–21, including the entire 2.9-kb coding sequence. Mutations found by cDNA sequencing, which were in exons 9, 13, and 17, were confirmed by genomic sequencing. In addition, a subset of heterozygous exon 11 mutations were evaluated at the cDNA level, to determine whether both the wild-type and mutant alleles were expressed.

Genomic DNA Sequencing. Genomic DNAs were isolated from frozen GIST specimens by the use of NaOH boiling preps with organic extractions. DNAs were isolated from paraffin sections of nonneoplastic companion tissues (evaluated to exclude the possibility of constitutional polymorphisms), using standard proteinase K digestion methods. Primers were chosen using the Whitehead Genome Center Primer3 software, and all primers were numbered according to c-*KIT* genomic sequence GenBank number U63834. Primers for amplification of exon 9 and exons 12–13 were as described (25). Primers for amplification of exons 10–11 were KIT I9–132F (5′-TCTGAGACTCACATAGCTTTGCATCC) and KIT I11 + 90R (5′-CACAGAAAACTCATTGTTTCAGGTGG), whereas primers for amplification of exon 17 were KIT I16–175F (5′-TGAACATCATTCAAGGCGTACTTTTG) and KIT I17 + 51R (5′-TTGAAACTAAAAATCCTTTGCAGGAC). Cycle sequencing of exons 10–11 and exon 17 was with the same primers used for PCR. All *KIT* sequencing reactions were performed from forward and reverse directions and in duplicate, and all mutations were confirmed by PCR amplification of an independent DNA isolate. PCR cycling conditions, gel extraction, and cycle sequencing with incorporation of ABI BigDye terminators were as described previously (25). Sequences were analyzed using ABI 310 or 377 automated sequencing machines.

cDNA Sequencing. RNAs were isolated from frozen tissue, and cDNAs were synthesized using avian myeloblastosis virus reverse transcriptase as described previously (25). The 2.9-kb KIT coding sequence was PCR amplified as overlapping 1.2-kb fragments, and each of these fragments was gel purified and cycle sequenced in total using three primers for forward and reverse reads. Details of the oligonucleotide primers are available by request (jfletcher@partners.org). Alignments and mutation scanning were performed using Sequence Navigator (Applied Biosystems) and BLAST (National Center for Biotechnology Information) software.

KIT Tyrosine Phosphorylation Analyses. Snap-frozen tumor specimens sufficient for preparation of cell lysates were available for 25 GISTs (including 3 benign, 4 borderline, and 19 malignant). These were ground to powder over liquid nitrogen and then resuspended in lysis buffer [1% NP40, 50 mmol/l Tris (pH 8.0), 100 mmol/l sodium fluoride, 30 mmol/l sodium PP_i, 2 mmol/l sodium molybdate, 5 mmol/l EDTA, 2 mmol/l sodium vanadate, and 10 μg/ml phenylmethylsulfonyl fluoride]. The lysates were rocked for 30 min at 4°C and then centrifuged to remove insoluble material. Supernatant protein concentrations were determined using the Bio-Rad MMT assay, and KIT was then immunoprecipitated from 500 μg of lysate, using a polyclonal antibody C-19 (Santa Cruz Biotechnology, Santa Cruz, CA) and Sepharose-protein A beads (Zymed Laboratories, South San Francisco, CA) as described previously (26). The immunoprecipitates were separated by gel electrophoresis and blotted to Protran 5 nitrocellulose membranes (Schleicher and Schuell, Keene, NH) and then stained with PY99 phosphotyrosine monoclonal antibody (Santa Cruz Biotechnology) with chemiluminescence detection. The blots were then stripped and restained with a second KIT polyclonal antibody A-4052 (Dako). Negative controls for KIT tyrosine phosphorylation included a frozen seminoma and a Ewing's sarcoma cell line (EWS794), which were characterized by high-level KIT expression but which lacked detectable KIT phosphorylation.

Results

Immunohistochemical analyses revealed strong and diffuse KIT (CD117) expression in each of the 48 GISTs, whereas CD34 was expressed in 38 cases. Nineteen GISTs expressed smooth muscle actin, which was generally focal, one had focal desmin, and none expressed S-100 protein.

A combination of genomic and cDNA sequencing revealed *KIT* mutations in 44 of 48 GISTs. *KIT* mutational status did not correlate with histological subtype nor with morphologically determined biological potential. *KIT* mutants were found in each of 10 benign GISTs, in 8 of 10 borderline GISTs, and in 26 of 28 malignant GISTs. Furthermore, *KIT* mutants were found in 31 of 32 spindle cell GISTs, 10 of 13 mixed histology GISTs, and 3 of 3 epithelioid GISTs. Most GISTs contained exon 11 mutations (juxtamembrane region, $n = 34$; 71%), whereas the remaining mutants were in exon 9 (extracellular region, $n = 6$, 13%), exon 13 (first lobe of the split kinase domain, $n = 2$, 4%), and exon 17 (phosphotransferase domain, $n = 2$, 4%). All mutations, whether involving bp substitutions, deletions, or duplications, preserved the open reading frame. The exon 11 mutations included missense, in-frame deletions, and in-frame duplications (Fig. 1). The exon 9 and 13 mutations, which have been reported previously (25), resulted in alanine-tyrosine duplication and lysine to glutamate substitution, respectively. The two exon 17 mutations resulted in substitution of either lysine or histidine for asparagine 822. Four GISTs had no identifiable *KIT* sequence alteration. Absence of apparent mutations, in these four tumors, was determined by genomic sequencing of exons 9–17 and by cDNA sequencing of exons 1–21 on two independent DNA and RNA isolates, respectively, from each of the cases. In addition, none of the four mutation-negative GISTs displayed aberrant genomic restriction fragments when evaluated by Southern blotting using a *KIT* cDNA probe encompassing the entire coding sequence (data not shown). Thus, 44 of 48 GISTs in this series (92%) possessed demonstrable KIT mutations.

Immunoblotting analyses showed KIT expression and tyrosine phosphorylation in each of 25 GISTs (Fig. 2). The 25 tumors evaluated in these studies included 15 of the 34 GISTs with exon 11 (juxtamembrane region) mutation, 2 of the 6 GISTs with exon 9 (extracellular region) mutation, and each of the GISTs with exon 13, exon 17, or no detectable mutation. There was no difference in the level of KIT expression and KIT tyrosine phosphorylation between the *KIT* wild-type and *KIT* mutant GISTs.

Discussion

The studies reported herein reveal oncogenic *KIT* mutations in 44 of 48 GISTs (92%), including each of 10 GISTs that were benign histologically. These findings differ substantially from those in prior series, where the reported frequency of *KIT* mutations ranged from 21 to 57% (9–12). As discussed below, there are several explanations for varying frequencies of KIT mutations in the different series. Chief among these is that previous series, save for the initial report of *KIT* mutations in 5 of 6 GISTs (8), have examined only exon 17 and/or exon 11. Our analyses of the entire 21-exon *KIT* coding sequence revealed recurring mutations in exons 9, 13, and 17. Individually, each of these regions is affected in substantially fewer GISTs than is exon 11. Collectively, however, the non-exon 11 mutations account for a substantial number (23%) of the overall mutations in this series. We also note that KIT activation, as manifested by receptor tyrosine

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                    550         560         570         580
                    ↓           ↓           ↓           ↓
[1]  TYKYLQKPMYEVQGKVVEEINGNNYVYIDPTQLPYDHKWEFPRNR
[2]  TYKYLQKPMYEVQRKVVEEINGNNYVYIDPTQLPYDHKWEFPRNR
[3]  TYKYLQKPMYEVQWKDVEEINGNNYVYIDPTQLPYDHKWEFPRNR
[1]  TYKYLQKPMYEVQWKVEEEINGNNYVYIDPTQLPYDHKWEFPRNR
[1]  TYKYLQKPMYEVQWKVGEEINGNNYVYIDPTQLPYDHKWEFPRNR
[1]  TYKYLQKPMYEVQWKVVEEINGNNYVYIDPTQPPYDHKWEFPRNR
[1]  TYKYLQKH---VQWKVVEEINGNNYVYIDPTQLPYDHKWEFPRNR
[1]  TYKYLQKPI---QWKVVEEINGNNYVYIDPTQLPYDHKWEFPRNR
[1]  TYKYLQKPT------VVEEINGNNYVYIDPTQLPYDHKWEFPRNR
[1]  TYKYLQKPMYEVQE--VEEINGNNYVYIDPTQLPYDHKWEFPRNR
[1]  TYKYLQKPMYEVQT--VEEINGNNYVYIDPTQLPYDHKWEFPRNR
[1]  TYKYLQKPMYEVQS-VVEEINGNNYVYIDPTQLPYDHKWEFPRNR
[1]  TYKYLQKPMYEVQS----------------TQLPYDHKWEFPRNR
[1]  TYKYLQKPMYEVQWK--ED-------YIDPTQLPYDHKWEFPRNR
[2]  TYKYLQKPMYEVQWNPVVEEINGNNYVYIDPTQLPYDHKWEFPRNR
[1]  TYKYLQK-----QWKVVEEINGNNYVYIDPTQLPYDHKWEFPRNR
[1]  TYKYLQK------WKVVEEINGNNYVYIDPTQLPYDHKWEFPRNR
[1]  TYKYLQKPM------VVEEINGNNYVYIDPTQLPYDHKWEFPRNR
[1]  TYKYLQKPMYE------EEINGNNYVYIDPTQLPYDHKWEFPRNR
[2]  TYKYLQKPMYEVQ--VVEEINGNNYVYIDPTQLPYDHKWEFPRNR
[1]  TYKYLQKPMYEVQW-------GNNYVYIDPTQLPYDHKWEFPRNR
[1]  TYKYLQKPMYEVQWK--EEINGNNYVYIDPTQLPYDHKWEFPRNR
[3]  TYKYLQKPMYEVQWKV-EEINGNNYVYIDPTQLPYDHKWEFPRNR
[1]  TYKYLQKPMYEVQWKVVEEINGNNYV-------PYDHKWEFPRNR
[1]  TYKYLQKPMYEVQWKVVEEINGNNYVYIDPTQLPYDHKWEFLPYDHKWEFPRNR
[1]  TYKYLQKPMYEVQWKVVEEINGNNYVYIDPTQLPYDHKWEFPLPYDHKWEFPPRNR
[1]  TYKYLQKPMYEVQWKVVEEINGNNYVYIDPTQLPYDHKWEFPRNRDPTQLPYDHKWEFPRNR
c-kit TYKYLQKPMYEVQWKVVEEINGNNYVYIDPTQLPYDHKWEFPRNR
v-kit ----------------EEINGNN--YIDPTQLPYDHKWEFPRNR
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Fig. 1. Predicted KIT juxtamembrane region sequence alterations in 34 GISTs with exon 11 genomic mutations. Amino acid substitutions are *boxed*, deletions are indicated using *dashes*, and insertions/duplications are *underlined*. The number of tumors with each particular mutation are indicated in *brackets* to the *right* of the sequences. Wild-type human (*c-kit*) and oncogenic viral (*v-kit*) sequences are shown at *bottom*.

phosphorylation, is a general phenomenon in GISTs. KIT receptor activation was a consequence of the GIST *KIT* mutations, irrespective of which domain—extracellular, juxtamembrane or kinase—contained the mutation. Indeed, as discussed below, KIT tyrosine phosphorylation was prominent even in the 4 GISTs that lacked demonstrable *KIT* sequence alterations. These studies are the first to demonstrate constitutive KIT phosphorylation in GIST cells, *in vivo*, and they reveal that oncogenic KIT mutations are early transforming events of relevance in both benign and malignant GISTs.

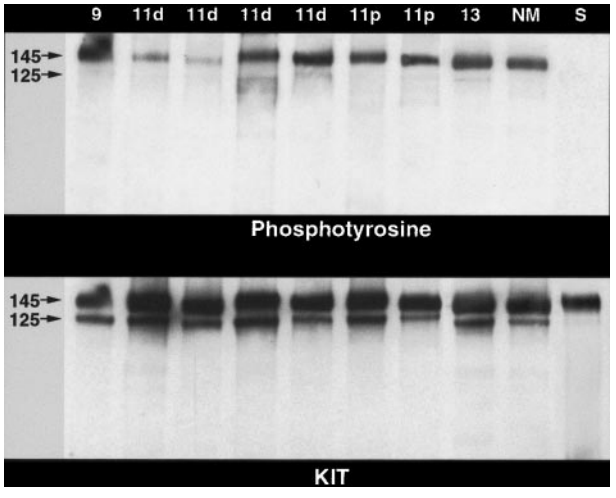

Fig. 2. Primary GIST cell lysates were immunoprecipitated with polyclonal anti-KIT, Western blotted, stained for phosphotyrosine (*top*), and then stripped and restained for KIT (*bottom*). *Lanes 9, 11d, 11p, 13,* and *NM,* GISTs with exon 9 mutation, exon 11 deletion mutation, exon 11 point mutation, exon 13 mutation, and no detectable mutation, respectively. *Lane S,* a non-GIST control (seminoma) that expresses nonphosphorylated KIT. *Arrows*, M_r 125,000 and M_r 145,000 KIT proteins, the M_r 145,000 form being the mature KIT protein that is fully glycosylated. Only the M_r 145,000 mature form is substantially tyrosine phosphorylated.

The generalized nature of KIT oncogenic activation and the evidence for a key KIT role in GIST pathogenesis are supported by immunohistochemical correlations and developmental biology evidence as follows: (*a*) KIT expression, as determined by immunohistochemistry, is diffuse and strong in virtually all GISTs (1, 27); (*b*) KIT is not down-regulated when GISTs progress to higher histological grade and/or metastasize. This situation contrasts with that in melanocytic lesions, where KIT expression is prominent in premalignant tumors but diminished or absent in malignant melanoma (28, 29); (*c*) experimental models in which KIT function is abrogated by inactivating mutations or immunological interventions (7) show that KIT is requisite for differentiation and proliferation of the ICC. It follows that KIT function might be equally vital for GIST cell survival, inasmuch as GISTs are thought to arise from progenitors in the ICC lineage (1–4); (*d*) *KIT* oncogenes are found in most or all tumor cells in GISTs and are thereby early, and potentially initiating, tumorigenic events in these neoplasms.[4] In sum, both developmental biology and tumor studies argue that KIT activation is pivotal in the pathogenesis of GISTs.

In the present study, we evaluated KIT oncogenic activation by a comprehensive sequence-based mutational analysis and by phosphotyrosine blotting assays. Mutations were identified in 44 of 48 GISTs, and each of these mutations preserved the *KIT* open reading frame and was therefore predicted to encode a functional KIT protein. This expectation was borne out by the phosphotyrosine immunoblotting studies (Fig. 2), which demonstrated highly phosphorylated M_r ~145,000 mature KIT proteins in each of 25 GISTs. Notably, KIT phosphorylation was always demonstrated, irrespective of which domain—extracellular, juxtamembrane, TK1, or TK2—contained the oncogenic mutation. It is also interesting that KIT was highly phosphorylated even in those GISTs ($n = 4$) that lacked demonstrable sequence mutations. There are several alternative mechanisms that might account

[4] B. Rubin, M. Lux, and J. A. Fletcher, unpublished data.

for KIT activation in the mutation-negative GISTs. One possibility is a mutational mechanism, such as a deletion or point mutation causing mispriming of one or more PCR primers, undetected by the genomic sequencing, cDNA sequencing, and Southern blotting assays used herein. Another possibility is KIT overexpression, brought about, for example, by transcriptional up-regulation or defective protein processing. This possibility is unlikely because neither paraffin section immunohistochemistry nor frozen tumor Western blotting showed more KIT protein expression in the mutation-negative GISTs compared with the rest of the group. Other possibilities, which remain to be evaluated, include inactivation of KIT-inhibitory phosphatases, up-regulation of the KIT ligand (stem cell factor), and KIT heterodimerization with other activated receptor-tyrosine kinase proteins. In sum, our data are in keeping with a potentially universal role for KIT activation in GIST pathogenesis. In turn, these findings suggest that KIT activation is requisite for neoplastic behavior in most GISTs. It will be intriguing to determine whether the KIT oncoproteins, in GISTs, signal differently from native KIT, as does the D816V KIT mutant in mastocytosis (30). Our preliminary studies suggest that this is indeed the case.[5]

The evidence reported herein is of substantial clinical relevance, particularly in underscoring KIT activation as a ubiquitous oncogenic pathway in GISTs. Novel therapies, potentially involving inhibition of KIT kinase function, would represent a major clinical advance in this disease (31). The KIT/ABL/PDGFR kinase inhibitor, STI571 (Gleevec), is one such therapeutic option, and we have determined that STI571 inhibits oncogenic KIT activation and GIST cell proliferation *in vitro* and *in vivo* (32, 33).

In summary, we have shown oncogenic *KIT* gene mutations in 44 of 48 GISTs, and we found biochemical KIT activation in each of 25 GISTs. These data implicate KIT activation as a central event in the pathogenesis of GISTs and suggest the possibility that activated KIT might serve as a universal therapeutic target in GISTs. This observation is of substantial clinical relevance, particularly given the recent development of effective KIT inhibitors (33), and in light of the fact that GISTs are invariably nonresponsive to conventional chemotherapies and radiation therapy.

Acknowledgments

We thank Joseph M. Corson and the pathology residents, fellows, and staff at Brigham and Women's Hospital for invaluable discussions and for assistance with specimen processing.

References

1. Kindblom, L. G., Remotti, H. E., Aldenborg, F., and Meis-Kindblom, J. M. Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am. J. Pathol., *152:* 1259–1269, 1998.
2. Sircar, K., Hewlett, B. R., Huizinga, J. D., Chorneyko, K., Berezin, I., and Riddell, R. H. Interstitial cells of Cajal as precursors of gastrointestinal stromal tumors. Am. J. Surg. Pathol., *23:* 377–389, 1999.
3. Sakurai, S., Fukasawa, T., Chong, J. M., Tanaka, A., and Fukayama, M. Embryonic form of smooth muscle myosin heavy chain (SMemb/MHC-B) in gastrointestinal stromal tumor and interstitial cells of Cajal. Am. J. Pathol., *154:* 23–28, 1999.
4. Robinson, T. L., Sircar, K., Hewlett, B. R., Chorneyko, K., Riddell, R. H., and Huizinga, J. D. Gastrointestinal stromal tumors may originate from a subset of CD34-positive interstitial cells of Cajal. Am. J. Pathol., *156:* 1157–1163, 2000.
5. Kluppel, M., Huizinga, J. D., Malysz, J., and Bernstein, A. Developmental origin and Kit-dependent development of the interstitial cells of Cajal in the mammalian small intestine. Dev. Dyn., *211:* 60–71, 1998.
6. Torihashi, S., Nishi, K., Tokutomi, Y., Nishi, T., Ward, S., and Sanders, K. M. Blockade of kit signaling induces transdifferentiation of interstitial cells of Cajal to a smooth muscle phenotype. Gastroenterology, *117:* 140–148, 1999.
7. Huizinga, J. D., Thuneberg, L., Kluppel, M., Malysz, J., Mikkelsen, H. B., and Bernstein, A. *W/kit* gene required for interstitial cells of Cajal and for intestinal pacemaker activity. Nature (Lond.), *373:* 347–349, 1995.
8. Hirota, S., Isozaki, K., Moriyama, Y., Hashimoto, K., Nishida, T., Ishiguro, S., Kawano, K., Hanada, M., Kurata, A., Takeda, M., Muhammad Tunio, G., Matsuzawa, Y., Kanakura, Y., Shinomura, Y., and Kitamura, Y. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science (Wash. DC), *279:* 577–580, 1998.
9. Ernst, S. I., Hubbs, A. E., Przygodzki, R. M., Emory, T. S., Sobin, L. H., and O'Leary, T. J. KIT mutation portends poor prognosis in gastrointestinal stromal/smooth muscle tumors. Lab. Investig., *78:* 1633–1636, 1998.
10. Moskaluk, C. A., Tian, Q., Marshall, C. R., Rumpel, C. A., Franquemont, D. W., and Frierson, H. F., Jr. Mutations of c-kit JM domain are found in a minority of human gastrointestinal stromal tumors. Oncogene, *18:* 1897–1902, 1999.
11. Lasota, J., Jasinski, M., Sarlomo-Rikala, M., and Miettinen, M. Mutations in exon 11 of c-Kit occur preferentially in malignant *versus* benign gastrointestinal stromal tumors and do not occur in leiomyomas or leiomyosarcomas. Am. J. Pathol., *154:* 53–60, 1999.
12. Taniguchi, M., Nishida, T., Hirota, S., Isozaki, K., Ito, T., Nomura, T., Matsuda, H., and Kitamura, Y. Effect of c-kit mutation on prognosis of gastrointestinal stromal tumors. Cancer Res., *59:* 4297–4300, 1999.
13. Fleischman, R. A. From white spots to stem cells: the role of the Kit receptor in mammalian development. Trends Genet., *9:* 285–290, 1993.
14. Russell, E. S. Hereditary anemias of the mouse: a review for geneticists. Adv. Genet., *20:* 357–459, 1979.
15. Kitamura, Y., and Go, S. Decreased production of mast cells in S1/S1d anemic mice. Blood, *53:* 492–497, 1979.
16. Isozaki, K., Hirota, S., Nakama, A., Miyagawa, J., Shinomura, Y., Xu, Z., Nomura, S., and Kitamura, Y. Disturbed intestinal movement, bile reflux to the stomach, and deficiency of c-kit-expressing cells in Ws/Ws mutant rats. Gastroenterology, *109:* 456–464, 1995.
17. Furitsu, T., Tsujimura, T., Tono, T., Ikeda, H., Kitayama, H., Koshimizu, U., Sugahara, H., Butterfield, J. H., Ashman, L. K., and Kanayama, Y. Identification of mutations in the coding sequence of the proto-oncogene c-*kit* in a human mast cell leukemia cell line causing ligand- independent activation of c-kit product. J. Clin. Investig., *92:* 1736–1744, 1993.
18. Tsujimura, T., Furitsu, T., Morimoto, M., Isozaki, K., Nomura, S., Matsuzawa, Y., Kitamura, Y., and Kanakura, Y. Ligand-independent activation of c-kit receptor tyrosine kinase in a murine mastocytoma cell line P-815 generated by a point mutation. Blood, *83:* 2619–2626, 1994.
19. Tsujimura, T., Furitsu, T., Morimoto, M., Kanayama, Y., Nomura, S., Matsuzawa, Y., Kitamura, Y., and Kanakura, Y. Substitution of an aspartic acid results in constitutive activation of c-kit receptor tyrosine kinase in a rat tumor mast cell line RBL-2H3. Int. Arch. Allergy Immunol., *106:* 377–385, 1995.
20. Tsujimura, T., Morimoto, M., Hashimoto, K., Moriyama, Y., Kitayama, H., Matsuzawa, Y., Kitamura, Y., and Kanakura, Y. Constitutive activation of c-kit in FMA3 murine mastocytoma cells caused by deletion of seven amino acids at the juxtamembrane domain. Blood, *87:* 273–283, 1996.
21. Nakata, Y., Kimura, A., Katoh, O., Kawaishi, K., Hyodo, H., Abe, K., Kuramoto, A., and Satow, Y. c-kit point mutation of extracellular domain in patients with myeloproliferative disorders. Br. J. Haematol., *91:* 661–663, 1995.
22. Tian, Q., Frierson, H. F., Jr., Krystal, G. W., and Moskaluk, C. A. Activating c-*kit* gene mutations in human germ cell tumors. Am. J. Pathol., *154:* 1643–1647, 1999.
23. Nakahara, M., Isozaki, K., Hirota, S., Miyagawa, J., Hase-Sawada, N., Taniguchi, M., Nishida, T., Kanayama, S., Kitamura, Y., Shinomura, Y., and Matsuzawa, Y. A novel gain-of-function mutation of c-*kit* gene in gastrointestinal stromal tumors. Gastroenterology, *115:* 1090–1095, 1998.
24. Newman, P. L., Wadden, C., and Fletcher, C. D. Gastrointestinal stromal tumours: correlation of immunophenotype with clinicopathological features. J. Pathol., *164:* 107–117, 1991.
25. Lux, M. L., Rubin, B. P., Biase, T. L., Chen, C. J., Maclure, T., Demetri, G., Xiao, S., Singer, S., Fletcher, C. D., and Fletcher, J. A. KIT extracellular and kinase domain mutations in gastrointestinal stromal tumors. Am. J. Pathol., *156:* 791–795, 2000.
26. Lawrence, B., Perez-Atayde, A., Hibbard, M. K., Rubin, B. P., Dal Cin, P., Pinkus, J. L., Pinkus, G. S., Xiao, S., Yi, E. S., Fletcher, C. D., and Fletcher, J. A. *TPM3-ALK* and *TPM4-ALK* oncogenes in inflammatory myofibroblastic tumors. Am. J. Pathol., *157:* 377–384, 2000.
27. Miettinen, M., Sobin, L. H., and Sarlomo-Rikala, M. Immunohistochemical spectrum of GISTs at different sites and their differential diagnosis with a reference to CD117 (KIT). Mod. Pathol., *13:* 1134–1142, 2000.
28. Ohashi, A., Funasaka, Y., Ueda, M., and Ichihashi, M. c-KIT receptor expression in cutaneous malignant melanoma and benign melanotic naevi. Melanoma Res., *6:* 25–30, 1996.
29. Montone, K. T., van Belle, P., Elenitsas, R., and Elder, D. E. Proto-oncogene c-*kit* expression in malignant melanoma: protein loss with tumor progression. Mod. Pathol., *10:* 939–944, 1997.
30. Chian, R., Young, S., Danilkovitch-Miagkova, A., Ronnstrand, L., Leonard, E., Ferrao, P., Ashman, L., and Linnekin, D. Phosphatidylinositol 3 kinase contributes to the transformation of hematopoietic cells by the D816V c-Kit mutant. Blood, *98:* 1365–1373, 2001.
31. Tuveson, D. A., and Fletcher, J. A. Signal transduction pathways in sarcoma as targets for therapeutic intervention. Curr. Opin. Oncol., *13:* 249–255, 2001.
32. Tuveson, D. A., Willis, N. A., Jacks, T., Griffin, J. D., Singer, S., Fletcher, C. D., Fletcher, J. A., and Demetri, G. D. STI571 inactivation of the gastrointestinal stromal tumor c-KIT oncoprotein: biological and clinical implications. Oncogene, *20:* 5054–5058, 2001.
33. Joensuu, H., Roberts, P. J., Sarlomo-Rikala, M., Andersson, L. C., Tervahartiala, P., Tuveson, D., Silberman, S., Capdeville, R., Dimitrijevic, S., Druker, B., and Demetri, G. D. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. N. Engl. J. Med., *344:* 1052–1056, 2001.

[5] A. Duensing and J. Fletcher, unpublished data.

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Cancer Res 2001;61:8118-8121.