Ligand-Independent Activation of c-kit Receptor Tyrosine Kinase in a Murine Mastocytoma Cell Line P-815 Generated by a Point Mutation

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The c-kit proto-oncogene encodes a receptor tyrosine kinase that is known to play a crucial role in hematopoiesis, especially in mast cell growth and differentiation. Although a number of dominant loss-of-function mutations of c-kit gene have been well characterized in mice, rats, and humans, little is known about the c-kit mutations contributing to ligand-independent activation of the c-kit receptor tyrosine kinase (KIT). In a murine mastocytoma cell line, P-815, KIT has been found to be constitutively phosphorylated on tyrosine and activated in a ligand-independent manner. Sequencing of the whole coding region of c-kit cDNA showed that c-kit cDNA of P-815 cells carries a point mutation in codon 814, resulting in amino acid substitution of Tyr for Asp. Murine wild-type c-kit cDNA and mutant-type c-kit cDNA encoding Tyr in codon 814 were expressed in cells of a human embryonic kidney cell line, 293T. In the transfected cells, mutant-form KIT<sup>Tyr814</sup> was strikingly phosphorylated on tyrosine and activated in immune complex kinase reaction regardless of stimulation with a ligand for KIT (stem cell factor), whereas tyrosine phosphorylation and activation was barely detectable in wild-form KIT. The data presented here provide evidence for a novel activating mutation of c-kit gene that might be involved in neoplastic growth or oncogenesis of some cell types, including mast cells.

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MATERIALS AND METHODS

Cell lines, cultured mast cells (CIMs), and mice. The P-815 cell line was originally established from a mastocytoma of a DBA/2 mouse by Donn and Potter, and was supplied by the Japanese Cancer Research Bank (Tokyo, Japan). P-815 cells were adapted to grow in Dulbecco's modification of Eagle's medium (DMEM; Flow Laboratories, Irvine, UK) supplemented with 10% fetal calf serum (FCS; Nippon Bio-Supp Center, Tokyo, Japan). The NIH/3T3 fibroblast cell line was generously provided by Dr S.A. Aaranson (National Cancer Institute, Bethesda, MD). The WC-SI/SI-3T3-fibroblasts and NIH/3T3 fibroblast line was established in our laboratory, as described previously. A human embryonic kidney cell line, 293T, was provided by Dr D. Baltimore (Rockefeller University, New York, NY), and was derived from human embryonic kidney cells transformed by DNA from human adenovirus type 5. 293T cells were maintained in DMEM supplemented with 10% FCS. To obtain CIMs, normal C57BL/6 +/- mice of 5 days of age were killed by overinhalation of ether. Spleens were removed and cells were suspended as described previously. Polyclonal rabbit antirat Ig antibody (DAKO Glostrup, Denmark) containing 2 x 10^6 spleen cells and 5 ml minimal essential medium (CMIM) were used for the preparation of the antigen-antibody complexes. The immune complexes were washed once with phosphate-buffered saline; twice with 0.5 mol/L LiCl and 50 mmol/L Tris-HCl, pH 7.4; and once with kinase buffer (10 mmol/L MocCl, 20 mmol/L Tris-HCl, pH 7.4) at 4°C. The immune complexes were then incubated in kinase buffer containing 1 mM of [gamma-P]-adenosine triphosphate (ATP, DuPont/NEN Research Products, Boston, MA; 10 mCi/mL) for 20 minutes at 25°C, washed, and separated by SDS-PAGE with 5% to 20% gradient polyacrylamide. The gel was dried and radioactive proteins were detected by autoradiography.

Detection of murine SCF transcript. The single-strand cDNAs were synthesized from total RNA of NIH/3T3 fibroblasts, WC-SI/SI-3T3 fibroblasts, and P-815 cells with a specific antisense primer (5'-AAGGTACACATGCTGGACAC-3') and of subjected to polymerase chain reaction (PCR) to amplify the 854 bp of DNA sequence between nucleotide 180 and 1033 of murine SCF cDNA using a specific sense primer (5'-TCTTATAGAACAGAAGACAC-3') and the antisense primer. Nucleotide numbers are based on the report by Anderson et al. PCR products were examined on 1% agarose gel with DNA digested with EcoRI and HindIII as a size marker.

Oligonucleotide primers. To isolate c-kit cDNA fragments, PCR was performed using six complementary oligonucleotide primers. The following oligonucleotide primers were used: primer 1, 5'-GAGGCTCAAGGTCGCGCAAC-3' (1 through 20); primer 2, 5'-AACCTAGGCCTGTGCGACC-3' (173 through 1796); primer 3, 5'-AGGGATATTGGAACAAATTG-3' (1707 through 1731); primer 4, 5'-CTTCGTTGCACTGCTGAGCACT-3' (2654 through 2678); primer 5, 5'-TAGGGCTACGCAGAACT-3' (2460 through 2481); primer 6, 5'-TTTCTATGTCCTGACATA-3' (3401 through 3419) (odd-numbered primers are sense primers and even-numbered primers are antisense primers). In addition to the above-mentioned oligonucleotide primers, the following oligonucleotide primers were also synthesized for the determination of nucleotide sequence: sense primers: primer 7, 5'-TCAATGAAATTGGTGAGAATC-3' (243 through 262); primer 8, 5'-CAAGGCTCCTGACACATCA-3' (529 through 548); primer 9, 5'-CATTACGCTTGGTCCACC-3' (714 through 733); primer 10, 5'-GTGGTAAATCAGACGGCC-3' (1039 through 1058); primer 11, 5'-GCCACCATGCTCCATACGAG-3' (2077 through 2093); primer 12, 5'-CAGATGTTCAAAAGAAGACAC-3' (2652 through 2670); primer 13, 5'-CGCTGTTGGTACCACT-3' (2869 through 2886); antisense primers: primer 14, 5'-GACGCTGACGTCC-3' (644 through 667); primer 15, 5'-CATATATACGCTGGTGTCG-3' (1066 through 1099); primer 16, 5'-GAGCCATGAGGTCAAGAAC-3' (1404 through 1423); primer 17, 5'-ATGGTCTCGTGGGACATT-3' (2527 through 2544) (the numbers in parentheses refer to the region of the sequence).

cDNA synthesis, isolation of PCR-amplified products, and sequencing of c-kit cDNA. Total RNA was extracted from P-815 cells and the cerebellums of DBA/2 +/- mice using the guanidium isothiocyanate method. From 5 μg of total RNA, the single-strand cDNA was synthesized using reverse-transcriptase and downstream antisense primers. cDNA was amplified by PCR using Taq DNA polymerase in 30 cycles of 1 minute of denaturation at 94°C, 2 minutes of annealing at 55°C, and 3 minutes of synthesis at 72°C. For the analysis of DNA sequence, products of reverse-transcriptase modification of PCR (RT-PCR) were gel-purified and then treated with T4 polynucleotide kinase and Klenow fragment of DNA polymerase I. The products were subcloned into the EcoRV site of Blue-Script I KS(-) (Stratagene, La Jolla, CA). Nucleotide sequence was determined by the method of Sanger et al using [3H]-α-deoxyxycytidine triphosphate (dCTP; DuPont/NEN; 10 mCi/mL).

Southern blotting analysis. Genomic DNAs were prepared from P-815 cells and the livers of DBA/2 +/- mice by the phenol extrac-
tion method. Purified DNAs were digested with Pst I, EcoRI, or Bgl II and then was subjected to 1% agarose electrophoresis. The mouse c-kit cDNA (nucleotides 1 to 3711) was used as a probe after labeling with [32P]-dCTP (DuPont/NEN; 10 mCi/mL) by random oligonucleotide priming.

Construction of transgene and transfection. The gene encoding murine wild-type c-kit cDNA (a generous gift of Dr S-I. Nishikawa, Kyoto University, Kyoto, Japan; hereafter called c-kit<sup>WT</sup> cDNA) was cloned into an EcoRI site of Bluescript II KS(−). To generate c-kit<sup>Y814F</sup> cDNA, a single transversion mutation (GC → TA) at nucleotide position 2468 was introduced into c-kit<sup>WT</sup> cDNA by exchanging the Nhe I-Acc I fragment (nucleotide 2458 to 2650) of c-kit<sup>WT</sup> cDNA for the corresponding fragment of c-kit<sup>Y814F</sup> cDNA obtained from murine wild-type c-kit cDNA (Kyoto University, Kyoto, Japan; hereafter called c-kit<sup>Y814F</sup> cDNA). The sequence was confirmed by DNA sequencing. The full coding sequence was confirmed by DNA sequencing. The full coding sequences of c-kit<sup>WT</sup> cDNA or c-kit<sup>Y814F</sup> cDNA were then released by HindIII and Sma I restriction digestion, isolated, and inserted into a HindIII-Sma I site of expression vector pSV2neo<sup>+</sup> to replace the neomycin resistance gene by c-kit<sup>WT</sup> cDNA or c-kit<sup>Y814F</sup> cDNA. As a result, c-kit<sup>WT</sup> cDNA or c-kit<sup>Y814F</sup> cDNA were flanked by an SV40 promoter sequence and a poly(A) site on the 3′ ends, respectively. The inserts were resequenced to confirm the orientation and the mutation within the expression vectors.

The expression vectors containing c-kit<sup>WT</sup> cDNA or c-kit<sup>Y814F</sup> cDNA were transfected into 293T cells by the calcium phosphate method as described previously. Moreover, both c-kit<sup>WT</sup> cDNA and c-kit<sup>Y814F</sup> cDNA were cotransfected into the cells to determine whether the conversion of Asp to Tyr in codon 814 is a dominant-positive mutation. After 40 hours of transfection, the cells were collected and used for the further analysis.

Metabolic labeling by [55S]-methionine. For metabolic labeling, cells were first incubated at 37°C for 2 hours in methionine-free Eagle’s minimal essential medium containing 5 mmol/L glucose, 1 mmol/L sodium pyruvate, and 10% dialyzed fetal bovine serum (Hazleton, Lenexa, KS). Cells were then incubated in the fresh medium containing [55S]-methionine (DuPont/NEN; 100 μCi/mL) for 4 hours. The labeled cells were collected and lysed in lysis buffer at 4°C for 20 minutes. Radiolabeled KIT were precipitated with ACK2 MoAb<sup>49</sup> and protein-G Sepharose beads. The immunoprecipitates were washed with lysis buffer and were subjected to SDS-PAGE with 5% to 20% gradient polyacrylamide. The gel was dried and radioactive proteins were detected by autoradiography.

RESULTS

Constitutive tyrosine phosphorylation and activation of KIT in P-815 cells. The expression of KIT in P-815 cells was first examined by flow cytometry using ACK2 MoAbs that recognize the extracellular domain of murine KIT. This analysis showed that KIT was apparently expressed on the cell surface of P-815 cells at an almost similar level to that of CMCs derived from normal C57BL/6-+/+ mice (+/+ CMCs) (Fig 1A).

We next examined the state of tyrosine phosphorylation of KIT in P-815 cells before and after stimulation with rmSCF. P-815 cells and +/+ CMCs were removed from growth factors for 4 hours and then stimulated with 100 ng/mL of rmSCF for 15 minutes at 37°C. KIT was then immunoprecipitated, and changes in tyrosine phosphorylation were detected by immunoblotting using a specific MoAb for phosphotyrosine. In accord with the previous finding of Rottapel et al.,<sup>23</sup> the immunoblotting analysis demonstrated that KIT was strikingly phosphorylated on tyrosine residues in P-815 cells before and after stimulation with rmSCF, whereas tyrosine phosphorylation of KIT in +/+ CMCs was observed only after treatment with rmSCF (Fig 1B). We further examined the autokinase activity of KIT in P-815 cells. KIT was immunoprecipitated from cell lysates before stimulation with rmSCF and was subjected to immune

Fig 1. Expression (A), tyrosine phosphorylation (B), and activation (C) of KIT in +/+ CMCs and P-815 cells. (A) Flow cytometric analysis of the surface binding of ACK2 MoAb to +/+ CMCs and P-815 cells. Cells were incubated in either ACK2 MoAb (-) or negative control antibody (-), washed, incubated with FITC-conjugated rabbit antirat Ig antibody, and analyzed on a FACScan. (B) To examine changes in tyrosine phosphorylation of KIT, KIT was immunoprecipitated with ACK2 MoAb from cell lysates before and 15 minutes after rmSCF stimulation, and immunoblot was performed with anti-phosphotyrosine MoAb. The mobilities of the mature (145 KD) and immature (125 KD) forms of KIT are indicated at right. (C) Immune complex kinase assay for autokinase activity of KITs in +/+ CMCs and P-815 cells. KIT was immunoprecipitated with a rabbit polyclonal antibody to the whole murine KIT from cell lysates before stimulation with rmSCF, and the immunoprecipitates were incubated in kinase buffer containing [γ-32P]ATP for 20 minutes at 25°C, washed, and separated by SDS-PAGE. Incorporation of 32P into KIT was visualized by autoradiography.
complex kinase assay. Although almost equal amounts of KIT were immunoprecipitated from both samples (data not shown), KIT of P-815 cells exhibited a striking tyrosine kinase activity before stimulation with rmSCF, whereas tyrosine kinase activity of KIT in +/+ CMC was barely detectable without rmSCF stimulation (Fig 1C).

**Absence of SCF transcripts in P-815 cells.** To determine whether the constitutive activation of KIT in P-815 cells was induced by autocrine mechanism, the expression of SCF transcripts was examined by RT-PCR analysis in P-815 cells and also in NIH/3T3 fibroblasts and WC-SI/SI/3T3-1 fibroblasts generated from genetically SCF-deficient mice, which were used as positive and negative controls, respectively. SCF transcripts were not observed in P-815 cells.

A single mutation in c-kit cDNA of P-815 cells. The constitutive tyrosine phosphorylation and activation of KIT in P-815 cells could be explained by mutation(s) in the c-kit proto-oncogene. Sequencing of the whole coding region of c-kit cDNA from P-815 cells showed a GC → TA transversion at nucleotide 2468, which resulted in amino acid substitution of Ala for Glu in codon 814. This point mutation was observed in cDNA clones generated from eight independent PCR products and was also confirmed by direct sequencing of PCR products amplified independently (Fig 3). Because the P-815 cell line was established from a mastocytoma that arose in a DBA/2 mouse, the sequence of c-kit cDNA obtained from the cerebellums of DBA/2-+/+ mice was examined. As shown in Fig 3, c-kit cDNA of DBA/2-+/+ mice was found to encode Asp, but not Tyr, in codon 814, in agreement with the published sequence of c-kit cDNA of a Balb/c mouse. These results suggested that the conversion of Asp to Tyr in codon 814 could be a mutation, but was not a strain-specific polymorphism.

In addition, three changes caused by the strain-specific polymorphism were observed in the c-kit cDNA sequence of P-815 cells: an AT → CG transversion at nucleotide 648 that resulted in the amino acid substitution of Ala for Glu in codon 207; a CG → TA transition at nucleotide 1618 that did not induce alteration of amino acids; and a GCG codon at nucleotides 2357 to 2359 changed to a GCC codon, leading to the change from Ala to Gly in codon 777 (data not shown).

**Southern blot analysis.** Although sequencing analyses clearly showed that c-kit cDNA of P-815 cells had a GC → TA transversion at nucleotide 2468 resulting in amino acid substitution of Tyr for Asp in codon 814, it was not clear whether the transversion was detectable on both alleles at the W/KIT locus. Therefore, Southern blot analysis was performed on genomic DNA of P-815 cells using the murine c-kit cDNA as a probe. When genomic DNAs obtained from livers of DBA/2-+/+ mice and P-815 cells were digested with Pst I, no difference in the pattern and intensity of bands was observed (Fig 4). However, when digested with either

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**Fig 2.** RT-PCR analysis of SCF transcripts in P-815 cells. Single-strand cDNA was synthesized from total RNA (5 μg of P-815 cells) and an 854-bp fragment (nucleotides 180 to 1033) of SCF cDNA was amplified by PCR. The products were subjected to 1% agarose gel with DNA digested with EcoRI and HindIII as a size marker. NIH/3T3 and WC-SI/SI/3T3-1 fibroblasts were used as positive and negative controls, respectively. SCF transcripts were not observed in P-815 cells.

**Fig 3.** Nucleotide sequence including the mutation site of c-kit cDNA obtained from P-815 cells. Shown are results from direct sequencing of DBA-+/+ mouse c-kit cDNA and P-815 cell c-kit cDNA from amino acid residues 809 to 819. P-815 cells were found to carry a mutation of c-kit gene at codon 814. Arrowheads indicate the mutation site of P-815 c-kit cDNA and the corresponding site of DBA-+/+ mouse c-kit cDNA.
Activating Mutation of Murine c-kit

EcoRI or Bgl II, genomic DNA of P-815 cells exhibited new bands that showed weaker signal magnitude than the surrounding bands and were not detectable in genomic cDNA of DBA/2-/+ mice (Fig 4). These results suggested that only a mutant allele with a GC→TA transversion at nucleotide 2468 was transcribed in P-815 cells, whereas another allele was not transcribed, possibly because of a gross rearrangement of the c-kit gene.

A single point mutation leads to constitutive activation of KIT. Human SCF is known to have little effect on cells expressing murine KIT, whereas murine SCF can exert its actions through interaction with both murine and human KITs.14,52 We therefore used human 293T cells as recipient cells for murine c-kit cDNA transfection to examine the causal role of the point mutation in constitutive activation of KIT; this combination could disregard the effect of human SCF on murine KIT, even if SCF might be produced from 293T cells after transfection. The c-kit<sup>Wld</sup> cDNA, c-kit<sup>TyrX14</sup> cDNA, or both were introduced into 293T cells; wild-form KIT and mutant-form KIT<sup>TyrX14</sup> were synthesized in 293T cells at an almost equal level (Fig 5A). To examine the tyrosine phosphorylation and activation of KIT and KIT<sup>TyrX14</sup> expressed in the transfected cells, they were immunoprecipitated and then subjected to immunoblotting with antiphosphotyrosine MoAb and also to immune complex kinase assay. As shown in Fig 5B, tyrosine phosphorylation of wild-form KIT was barely detected in the absence of rmSCF, and it was significantly augmented by the stimulation with SCF. In 293T cells transfected either with c-kit<sup>TyrX14</sup> cDNA alone or with both c-kit<sup>TyrX14</sup> cDNA and c-kit<sup>Wld</sup> cDNA, an abun-
dant tyrosine phosphorylation and a striking autokinase activity were observed regardless of rmSCF stimulation (Fig 5B and C). These results suggested that the Asp → Tyr mutation may lead not only to constitutive activation of KIT but also to an increase in the intrinsic kinase activity of KIT.

**DISCUSSION**

Binding of a ligand to the cognate RTK triggers a series of rapid events initiating a signal cascade that leads to mitogenesis, ie, receptor dimerization, RTK-tyrosyl autophosphorylation, activation of kinase, association of RTK with its substrates such as phosphatidylinositol 3'-kinase, and phosphorylation of other cellular proteins.10,11 Thus, RTKs are normally regulated by their ligands. In addition, RTKs have been reported to be constitutively activated by a point mutation, often by multiple mutations, as well as by deletions that affect the primary structure of the protein.10,11,26 Comparative structure analysis of oncogene v-kit and protooncogene c-kit showed that, in oncogene v-kit, N-terminal sequences, including the transmembrane domain, and C-terminal sequences are deleted, leaving a minimal kinase domain fused to 340 amino acids of feline leukemia virus gag.1,2 These results suggest that these structural alterations are likely to be involved in oncogenic activation of v-kit, and also implicate that additional sites for KIT activation may exist in post-oncogene c-kit. However, little is known about mutation(s) of post-oncogene c-kit that lead to constitutive, ligand-independent activation of KIT.

In this study, we have shown that KIT is phosphorylated on tyrosine residues and is activated in P-815 cells in a ligand-independent manner. These observations are consistent with the previous findings by Rottapel et al.33 Sequencing analysis of P-815 cells showed a point mutation resulting in amino acid substitution of Tyr for Asp in codon 814. To determine whether the point mutation was responsible for the constitutive tyrosine phosphorylation and activation of KIT in P-815 cells, c-kitTyr814 cDNA was transected into cells from a human embryonic kidney cell line, 293T. In the transfected 293T cells, mutant-form KITTyr814 was found to be strikingly phosphorylated on tyrosine and activated in the absence of rmSCF, whereas wild-form KIT was neither phosphorylated on tyrosine nor activated unless it was stimulated by rmSCF. These results indicate that the conversion of Asp to Tyr in codon 814 is an activating mutation and plays a causal role in constitutive, ligand-independent activation of KIT in P-815 cells.

Sequencing analysis showed that no cDNA clones obtained from P-815 cells demonstrated normal c-kit sequence. In light of the data on Southern blot analysis, a gross rearrangement of c-kit gene was considered to occur in one allele. These results suggested that the only mutated allele with the Asp → Tyr conversion was transcribed in P-815 cells, whereas the other allele was not transcribed because of the rearrangement. To determine whether the Asp → Tyr mutation is dominant-positive, mutant-form KITTyr814 was coexpressed with wild-form KIT. In 293T cells transfected either with c-kitTyr814 cDNA alone or with both c-kitTyr814 cDNA and c-kitTyr814 cDNA, an abundant tyrosine phosphorylation and a striking autokinase activity were observed, regardless of rmSCF stimulation. These results suggested that the Asp → Tyr conversion is a dominant-positive activating mutation.

An activating mutation in codon 816 of human KIT, corresponding to codon 814 of mouse KIT, was also detected in a human mast-cell leukemia cell line, HMC-1.32 We have recently shown that c-kit genes of HMC-1 cells were composed of a normal allele and a mutant allele with two point mutations in codon 560 (GTT → GTC) and codon 816 (GAC → GTC), resulting in intracelular amino acid substitutions of Gly for Val and Val for Asp, respectively. When mutant-type murine c-kit cDNAs encoding Gly560 and/or Val814, corresponding to human Gly560 and/or Val814, were expressed in 293T cells, mutant-form KITGly560,Val814 or KITVal814 was phosphorylated on tyrosine and activated at equally high levels even in the absence of rmSCF, whereas tyrosine phosphorylation and activation of mutant-form KITGly560,Val814 or wild-form KIT was modest or slight, respectively.32 These results indicated that a murine Asp814 → Val814 mutation, corresponding to a human Asp816 → Val816 mutation, may be a dominant-positive activating mutation. Taking these facts together, it is suggested that constitutive activation of KIT can result from a single amino-acid replacement of Asp by either Val or Tyr in codon 814, and that codon 814 of murine c-kit gene may be a “hot spot” for activating mutation.

Although the constitutive activation of KIT caused by the codon 814 mutations within the tyrosine kinase domain are dramatic, the oncogenic potential or the biologic effect of these mutations has yet to be clarified. This question is currently being examined by transfection of mutant-type c-kit cDNA into hematopoietic stem cells or factor-dependent hematopoietic cell lines, and these examinations could provide insights into the biologic significance of the mutations. However, it is important to note that the two types of cells bearing the activating mutations of c-kit gene are both neoplastic mast cells. Given the fact that KIT is implicated as an indispensable molecule for the proliferation and differentiation of mast cells,6,12,18,24,27,31 these results raise the possibility that the codon 814 mutations may be at least partially involved in neoplastic growth or oncogenesis of mast cells. The extensive surveys on de novo mast-cell neoplasms might be helpful to show whether the codon 814 mutations contribute to the development of mast-cell malignancies.

In addition to mast-cell malignancies, it has been suggested that KIT or c-kit mRNA has been detected in a variety of neoplastic cells. For example, we and others have reported that KIT is expressed in human leukemia cell lines of erythroid and megakaryocytic lineages, and also in most cases of human acute myeloblastic leukemia cells and some cases of chronic myelogenous leukemia in blast crisis.43,64,53-57 Also, KIT has been reported to be expressed in testicular germ cell tumors and small-cell lung cancers.20,29 It is therefore possible that, if present, the codon 814 mutation of c-kit might etiologically contribute to various malignancies other than those of mast cells.

Although the codon 814 mutations seem not only to constitutively activate KIT but also to increase the intrinsic kinase activity of KIT, the underlying mechanism(s) whereby the mutations lead to ligand-independent activation of KIT.
is not known at this time. However, it may be important to note that the amino acid sequence of the Asp<sup>814</sup> region in KIT tyrosine kinase domain is widely conserved in many other RTKs such as receptors for PDGF, CSF-1, insulin, and hepatic growth factor. Therefore, it is possible that the amino acid sequence of the Asp<sup>814</sup> region may have an essential role in the structure and function of many RTKs. If so, the conversion of Asp to either Tyr or Val in codon 814 might induce a conformational change of KIT, thereby leading to receptor dimerization that is thought to be essential for RTK activation. Also, it is possible that similar mutations in other RTKs might yield activating variants as well and participate in the development of a variety of malignancies. Further studies on c-kit mutants will not only provide important insight into the fundamental mechanism(s) underlying regulation of normal cell growth but will also provide further understanding of oncogenesis mediated by RTKs.

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Ligand-independent activation of c-kit receptor tyrosine kinase in a murine mastocytoma cell line P-815 generated by a point mutation

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