CMLS, Cell. Mol. Life Sci. 61 (2004) 2924–2931
1420-682X/04/232924-08
DOI 10.1007/s00018-004-4273-y

CMLS Cellular and Molecular Life Sciences

Kit as a human oncogenic tyrosine kinase

Y. Kitamura [a, *] **and S. Hirota** [b]

[a] Shionogi Pharmaceutical Company, 3-1-1 Futaba-cho, Toyonaka, Osaka 561-0825 (Japan), Fax: +81 6 6332 6385, e-mail: yukihiko.kitamura@shionogi.co.jp
[b] Department of Pathology, Medical School/Graduate School of Frontier Bioscience, Osaka University, Yamada-oka 2-2, Suita, Osaka 565-0871 (Japan)

Abstract. Signals through Kit receptor tyrosine kinase are essential for development of erythrocytes, melanocytes, germ cells, mast cells and interstitial cells of Cajal (ICCs). Mice and rats with a double gene dose of loss-of-function mutations of Kit show depletion of these cells. Although human homozygotes with loss-of-function mutations of Kit have not been reported, gain-of-function mutations of Kit result in development of tumors from mast cells, germ cells and ICCs in humans. The ICC tumors are called gastrointestinal stromal tumors (GISTs), and GISTs are a good target for the Kit inhibitor imatinib mesylate. The interrelationship between the type of Kit gain-of-function mutations and the therapeutic effect of imatinib mesylate has been well characterized in GISTs. Kit is interesting from both a biological and clinical viewpoint.

Key words. Kit; KitL; mast cell; germ cell; interstitial cell of Cajal; imatinib mesylate.

Introduction

By their remarkable phenotypes [1], mouse mutants at the W locus were known for a long time before the W locus was identified to encode Kit receptor tyrosine kinase [2, 3]. Although the v-kit gene was found to be an oncogene in cats [4], it took many years before the mutated Kit gene was demonstrated to be a cause of human tumors [5]. However, after the identification of the mutated Kit gene as a cause of human gastrointestinal stromal tumors (GISTs) [6], it took only 3 years for the first successful administration of a Kit inhibitor to a GIST patient [7, 8].

Structure and function of Kit

The Hardy-Zuckerman 4 feline sarcoma virus (HZ4-FeSV) was isolated from a feline fibrosarcoma by Besmer et al. [4]. The viral genome of HZ4-FeSV contains a new oncogene designated v-kit [4]. HZ4-FeSV appears to have been generated by transduction of feline c-kit sequences with feline leukemia virus. Kit encoded by the c-kit gene is a type III receptor tyrosine kinase and is structurally similar to platelet-derived growth factor receptors (PDGFRs) alpha and beta, CSF-1R and Flt-3 [2, 3, 9, 10]. These receptor tyrosine kinases have unique features: an extracellular (EC) domain made up of five immunoglobulin-like repeats, and a tyrosine kinase (TK) domain that is split into two domains (TK-I and TK-II) by an insert sequence of variable length. The structure and amino acid sequence of Kit are well preserved in humans, mice and rats. For many years after the discovery of the v-kit gene, it remained unclear whether Kit played a role in the development of human neoplasms. Now, activating mutations have been found in various human tumors [6, 11–13].

The W locus of mice was demonstrated to encode Kit [2, 3]. Various types of loss-of-function mutants have been reported at the W locus. Among them, double heterozygous mice of the W/W^v genotype are most frequently used. The W mutant allele encodes a truncated Kit without the transmembrane domain; as a result, the EC domain is not expressed on the cell surface [14, 15]. The W^v mutant allele is a point mutation at the TK-I domain, resulting in a remarkable decrease in TK activity [14]. W/W^v mice show five abnormalities due to the loss of Kit function. (i). Anemia due to hypoproduction of erythrocytes [1]; (ii) white coat color due to lack of melanocytes [1]. Melanocytes are also deficient in the inner ears of W^v/W^v

* Corresponding author.

mice, and this results in hearing difficulty [16]; (iii) sterility due to depletion of germ cells in both males and females [1]; (iv) depletion of mast cells [17]; (v) depletion of interstitial cells of Cajal (ICCs) [18]. Abnormalities 1–3 have been known for a long time. We found the depletion of mast cells in 1978 [17], and Maeda et al. [18] found depletion of ICCs in 1992.

We found a rat mutant at the c-kit locus [19]. The mutant allele, named Ws, has an in-frame deletion of 12 bp at the TK-II domain [19]. Ws/Ws rats showed symptoms similar to those of W/W^v mice [20]. However, some differences were observed. Severe anemia is present in suckling Ws/Ws rats, but it ameliorates with age, and only slight anemia is detectable in adult Ws/Ws rats [21]. Also, male and female Ws/Ws rats are fertile in spite of the decreased weights of gonads. In humans, heterozygous c-kit mutants have been identified [22]. They show the dominant white spotting that is similar to the coat color phenotype of W/+ heterozygous mice.

The Kit ligand (KitL) was identified [23]. Since KitL is encoded by the Sl locus of mice, homozygous or double heterozygous mutant mice at the W or Sl locus have the same phenotype. The most frequently used mutant mice of the Sl locus are of the Sl/Sl^d genotype. Sl/Sl^d mice show anemia, white coat color, sterility, depletion of mast cells and ICCs.

We found spontaneous development of forestomach papillomas [24] and antral ulcers [25] in W/W^v and Sl/Sl^d mutant mice at around the same time we discovered depletion of mast cells in these mice [17, 26]. Bile reflux from the duodenum to the stomach appeared to be a cause of the stomach lesions in mice [27]. However, the mechanism of the bile reflux remained unknown. Since the depletion of ICCs was identified as the fifth abnormality of W/W^v and Sl/Sl^d mice [18, 28, 29], and since ICCs regulate the peristaltic movement of the gastrointestinal (GI) tract [30], the bile reflux is now attributed to the depletion of ICCs. The bile reflux to the stomach was also observed in Ws/Ws rats [31].

Signaling pathways

Binding of dimerized KitL induces the dimerization of Kit [32]. This leads to the autophosphorylation of Kit on tyrosine and its association with substrates of various kinds. The phophatidylinositol-3-kinase (PI3K)/Akt system is one of the major pathways [33] (fig. 1). PI3K is composed of an 85-kDa regulatory subunit and a 110-kDa catalytic subunit. The 85-kDa regulatory subunit is associated with activated Kit through an SH2 domain, and then is phosphorylated on tyrosine. The 110-kDa catalytic subunit of the activated PI3K produces phosphatidylinositol-3,4-bisphosphate, which in turn is used for phosphorylation of Akt, a serine-threonine kinase. The activated Akt is involved in the inhibition of apoptosis of cells whose survival depends on Kit signaling. A tyrosine kinase inhibitor, imatinib mesylate blocks not only autophosphorylation of Kit but also phosphorylation of Akt [34]. Induction of apoptosis in Kit signaling-dependent cells by imatinib mesylate may be mediated through the inhibition of Akt activity.

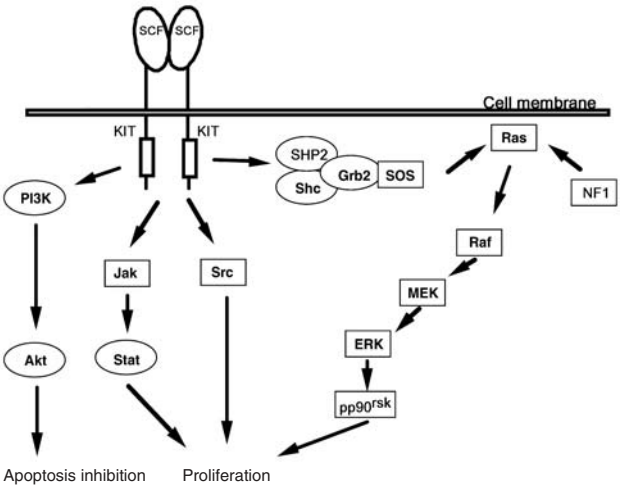

Figure 1. A scheme showing the signal transduction pathways of Kit. Dimerization and autophosphorylation of Kit result in cell proliferation and inhibition of apoptosis through several pathways, such as the PI3K/Akt system, the Ras/MAP kinase cascade, Src family members and the JAK/STAT system.

Another major signaling pathway is the Ras/MAP kinase cascade [33] (fig. 1). The activated Kit recruits SH-2-containing proteins such as Grb2, Shc and SHP2. Grb2 may bind Kit either directly or indirectly through interaction with Shc or SHP2. Since Grb2 is constitutively associated with Sos, a guanine nucleotide exchange factor, the recruitment of Grb2 to the activated Kit resultes in the colocalization of Sos and Ras and the subsequent activation of Ras. This promotes the interaction of Ras with Raf serine-threonine kinase and then the activation of MEK (a MAP kinase kinase). MEK phosphorylates ERK (a MAP kinase), and ERK phosphorylates a number of substrates, including $pp90^{rsk}$ [33]. Imatinib mesylate also inhibits the phosphorylation of ERK [34]. This process is considered to be due to blocking of the Kit signaling by this drug. Thus, both major signaling pathways of Kit, the PI3K/Akt system and the Ras/MAP kinase cascade, are inhibited by imatinib mesylate. Alternatively, Kit signaling may also activate JNK kinase, a MAP kinase activated in response to stress [35]. NF1, a GTPase activating protein, is considered to be involved in modulating Ras activation through Kit signaling [36]. NF1 deficiency that occurs in cases of neurofibromatosis may induce an increase in MAP kinase activity.

Pathways with JAK/STAT or with Src family members may be involved in Kit signaling [33] (fig. 1). Some studies showed the association of JAK2 with Kit and also its

activation by Kit signaling [37]. Other reports also indicated the increased activity of Lyn, a Src family member, by Kit signaling [38].

Tumors related to Kit abnormality

Mast cell neoplasms

In human mast cell leukemia cell line, HMC-1, Kit was constitutively phosphorylated on tyrosine, activated and then could associate with PI3K without addition of KitL. Furitsu et al. [5] found that the c-kit gene of HMC-1 cells was composed of a wild-type allele and a mutant allele with point mutations resulting in substitution of Val-560 to Gly in the juxtamembrane (JM) domain and Asp-816 to Val in the TK-II domain. Amino acid sequences in the region of the two mutations are completely conserved in mouse, rat and human Kit.

In order to determine the causal role of these mutations in constitutive activation, mutant Kit gene with the Val-560 to Gly mutation in the JM domain or with the Asp-816 to Val mutation in the TK-II domain was constructed and expressed in a human embryonic kidney cell line, 293T [5]. In the transfected cells, Kit with either mutation was abundantly phosphorylated on tyrosine and activated in the immune complex kinase reaction without the addition of KitL. Tsujimura et al. [39, 40] found the mutation corresponding to Asp-816 to Val of the human HMC-1 cell line in the P-815 mouse mastocytoma cell line (Asp-817 to Tyr) and the RBL-2H3 rat mast cell leukemia cell line (Asp-814 to Tyr). Both P-815 and RBL-2H3 cells showed constitutive activation of Kit without KitL.

Kit mutations were considered to induce the transformation of human mast cells. In fact, the Asp-816 mutations have been found in various types of mast cell neoplasms of adults, such as cutaneous mastocytosis, systemic mastocytosis, systemic mastocytosis associated with clonal hematologic non-mast cell lineage disorders and mast cell leukemia [11, 12, 41–43] (fig. 2). In humans, however, mast cell neoplasmas are more common in children than in adults. The Asp-816 mutations were rare in the cutaneous mastocytosis of children (urticaria pigmentosa) [41, 42]. Most cases of urticaria pigmentosa regress spontaneously before adolescence. In some cases of urticaria pigmentosa, loss-of-function mutations of Kit were found, but their etiological meanings are unknown [42]. When compared with dogs, mast cell neoplasms are rare even in children. In some dog mast cell neoplasms, internal tandem duplication was found in the JM domain of Kit [44]. This type of Kit mutation has not been reported in human mast cell neoplasms and other blood cell neoplasms, but this type of Flt-3 mutation is frequently found in human acute myeloid leukemias [45].

To examine the transformation potential of the Kit activation mutation, we used the murine interleukin-3 (IL-3)-dependent IC-2 mast cell line as a transfectant [46]. The IC-2 cells that had been established by Koyasu et al. [47] from murine cultured mast cell did not express Kit on the surface. The Asp-814 to Val or Val-559 to Gly murine type mutant Kit complementary DNA (cDNA) was introduced into IC-2 cells using a retrovirus vector. The mutant Kit expressed in IC-2 cells was constitutively phosphorylated on tyrosine and demonstrated kinase activity in the absence of KitL. IC-2 cells expressing mutant Kit showed factor-independent growth in suspension culture and produced tumors in nude athymic mice [46]. Introduction of the mutant Kit cDNA also resulted in transformation of the IL-3-dependent Ba/F3 murine pro-B cells [48].

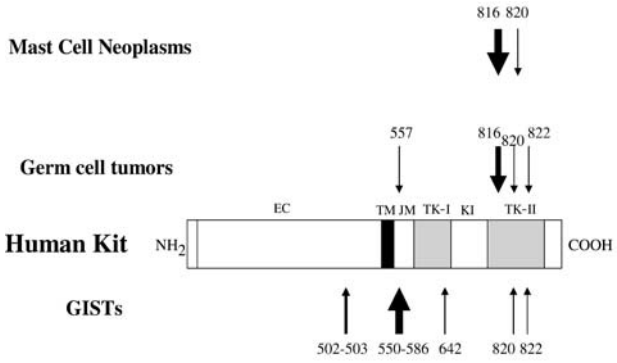

Figure 2. Kit mutations reported in human mast cell neoplasms, germ cell tumors and GISTs. EC, extracellular domain, TM, transmembrane domain; JM, juxtamembrane domain; TK-I, tyrosine kinase-I domain; KI, kinase insert; TK-II, tyrosine kinase-II domain. Sizes of arrows roughly parallel to the proportion of cases with each mutation.

The mechanisms of constitutive activation were different in the cases of Val-559 to Gly JM mutation and Asp-814 to Val TK-II mutation. This was shown by chemical cross-linking analysis [48]. A substantial fraction of phosphorylated Kit with Val-559 to Gly mutation dimerized, whereas phosphorylated Kit with Asp-814 to Val mutation did not. Tsujimura et al. [49] found another gain-of-function mutation at the JM domain of FMA3 murine mastocytoma cell line. The Kit cDNA of FMA3 cells carried an in-frame deletion of 21 bp. The FMA3-type Kit cDNA was introduced into IC-2 cells. The FMA3-type Kit was constitutively phosphorylated on tyrosine and activated. IC-2 cells expressing FMA3-type Kit grew in suspension culture without IL-3 and KitL, and became leukemic in nude athymic mice. Although the Val-559 to Gly mutation and FMA3-type mutation with 21-bp deletion were different in nature, their biological effects were comparable [48, 49].

Sporadic GISTs

Loss-of-function mutation of Kit resulted in depletion of mast cells [17], whereas gain-of-function mutation re-

sulted in mast cell neoplasms [5, 11, 12]. Since loss-of-function mutations of Kit caused depletion of ICCs [18], it seems reasonable that gain-of-function mutations might induce neoplasms of ICCs. However, when we started examining this possibility, the existence of ICC-derived tumors was not known. Therefore, we examined whether any mesenchymal tumors in the human GI tract express Kit using immunohistochemistry. Authentic leiomyomas and shwannomas did not express Kit, but most tumors designated GISTs did express it [6]. This suggested that GISTs originated from ICCs. Soon after the publication of our paper, Kindblom et al. [50] independently reported a similar result. Many GI tumors that were previously classified as leiomyomas and leiomyosarcomas express Kit, and consequently they are currently considered to be GISTs.

The whole coding region of Kit was obtained from six GISTs and sequenced. Mutations were observed in five out of six GISTs [6]. All mutations were detected in the JM domain, but not at the identical sites. We next examined whether the Kit mutations found in GISTs resulted in constitutive activation by means of transient introduction of mutant Kit cDNA into the 293T human embryonic kidney cell line. The Kit mutations found in GISTs showed constitutive tyrosine phosphorylation in 293T cells without KitL [6]. To investigate the biological consequences of mutant Kit, we introduced the Kit mutations found in human GISTs into mouse Kit cDNA and stably transfected it into the interleukin (IL)-3-dependent Ba/F3 murine pro-B cell line. Ba/F3 cells with the mutated murine Kit grew autonomously in nude mice [6].

Corless et al. [51] found mutated Kit in very small GISTs that were incidentally found at the time of surgical operations for other purposes. This suggests that the c-kit gene mutation is an early event of GIST development.

Mutations in the JM domain were most common in GISTs (~80%), and therefore were found first. Mutations in other domains were found thereafter: duplication of two particular amino acids in the EC domain (~5%) [52–54], and a point mutation at the TK-II domain (~2%) [55] (fig. 2). The site of TK-II mutation was different from that of the Asp-816 to Val mutation, which was found in human mast cell neoplasms.

In ~10% of GISTs, no Kit mutations were found even when the whole coding region was examined using fresh materials. Recently Heinrich et al. [56] and Hirota et al. [57] investigated the cause of GISTs without Kit mutations and found gain-of-function mutations of PDGFR-alpha in about one-third of GISTs without Kit mutations. Mutations were detected at both JM and TK-II domains. Interestingly, the Asp-842 of PDGFR-alpha corresponds to Asp-816 of Kit. As already mentioned, the Asp-816 mutation is common in human mast cell neoplasms but has not been reported in human GISTs. In other words, the particular Asp mutation in the TK-II domain is observed in PDGFR-alpha gene but not in Kit gene of human GISTs.

Familial GISTs

Most GISTs are sporadic, but we found three families with germline mutation of Kit and development of multiple GISTs [58–60]. First, Nishida et al. [58] found multiple GISTs in a 60-year-old Japanese woman who received two surgical operations due to intestinal obstruction. A nephew of this woman also suffered from multiple GISTs. Analysis of the family pedigree revealed that many family members suffered from intestinal obstruction that may be attributable to multiple GISTs. Although the GISTs of the 60-year-old woman and her nephew were benign, a niece of the woman died of malignant GIST that disseminated in the peritoneal cavity. DNA was extracted from GISTs and leukocytes of the woman and the nephew. An identical mutation was found in the JM domain of Kit, whereas this mutation was not detected in leukocytes obtained from other family members in whom GISTs were not observed [58]. The second family also showed the JM mutation [59], and the third family showed the mutation at Asp-820 of the TK-II domain [60].

In familial GISTs, remarkable hyperplasia of ICCs was observed in small and large intestines [59–62]. ICCs constitute one or two cell layers in the normal intestine, whereas hyperplasia with 10–20 cell layers was observed in familial GIST patients. Multiple GISTs develop from the hyperplasia. We examined the clonality of the hyperplastic lesions and GISTs in patients with familial GISTs, using random X chromosome inactivation in females [63]. The hyperplastic lesion was polyclonal, and each GIST derived from the hyperplastic lesion was monoclonal.

In addition to multiple GISTs, hyperpigmentation of the skin and/or mast cell neoplasms were reported in some families [58, 62, 64]. Most family members with multiple GISTs survive and can have offspring, and moreover, various Kit mutations cause the familial GISTs. Therefore, this disease entity may be a more common cancer syndrome than is presently supposed.

Sommer et al. [65] produced a mouse model for familial GISTs by a knock-in strategy introducing a JM domain mutation of Kit into the mouse genome. Patchy hyperplasia of ICCs is evident within the myenteric plexus of the entire GI tract, and neoplastic lesions indistinguishable from human GISTs were observed in the caecum of the mutant mice with high penetrance. These results demonstrate that constitutive Kit signaling is critical and sufficient for induction of the hyperplasia of ICCs and GISTs.

Germ cell tumors

Several researchers examined the presence of Kit mutation in human germ cell tumors [13, 66, 67]. Although the proportion of gain-of-function mutations of Kit was not so high as in the cases of mast cell neoplasms and GISTs, an appreciable proportion of testicular seminomas showed gain-of-function mutations of Kit. Most mutations were located at the TK-II domain as mast cell neoplasms (fig 2). Moreover, the Asp-816 mutation was most frequently found, as in the case of human mast cell neoplasms. However, Sakurai et al. [66] reported a case of seminoma with the gain-of-function mutation in the JM domain, as in the case of most GISTs. The gain-of-function mutation of Kit has not been reported in non-seminomatous germ cell tumors of testes [66]. Although the Asp-816 mutation in the TK-II domain was reported in an ovarian dysgerminoma [13], only a few reports described the interrelation between ovarian germ cell tumors and Kit.

Small cell lung cancer

An appreciable proportion of small cell lung cancers (SCLCs) expressed both Kit and KitL [68, 69]. This coexpression is considered to constitute a fundamental autocrine loop for growth of SCLC. Mutations of Kit have, however not been reported in SCLC, and its role in the pathogenesis of SCLC remains unresolved.

Specific drugs

Imatinib mesylate was initially developed at Ciba-Geigy (now Novartis) as a specific inhibitor of PDGFR [70]. Then it was found to be a potent inhibitor of Bcr-Abl [71]. In addition to the inhibitory effect of imatinib mesylate on PDGFR and Bcr-Abl, Buchdunger et al. [72] found the inhibitory effect on wild-type Kit. Some reports showed that imatinib mesylate also inhibited various type of mutated Kit found in GISTs [34, 73]. Before the publication of these data, treatment of the first GIST patient with imatinib mesylate began in Finland [7]. The patient's tumor expressed Kit and contained a JM domain mutation of Kit. The patient had progressive, widely metastatic tumors after failure of previous extensive therapy, including multiple surgical procedures and chemotherapy. Within a few weeks of starting daily oral administration of imatinib mesylate, the patient exhibited an objective clinical response that was maintained for more than 18 months. This encouraging result was published in 2001 [7]. A multicenter trial on advanced GISTs was done and reported in 2002 [74]. The US Food and Drug Administration has approved imatinib mesylate as effective therapy for advanced GIST patients.

The location of Kit and PDGFR-alpha mutations is related to the effectiveness of imatinib mesylate [75]. The partial response rate of the GISTs with JM domain mutations of Kit was 83.5%, whereas that of GISTs with the EC domain mutation of Kit was 47.8%. Although the number of the tested cases was small, GISTs with JM domain mutation of PDGFR-alpha responded to imatinib mesylate. GISTs with TK-II domain mutations of PDGFR-alpha and GISTs without any detectable Kit and PDGFR-alpha mutations did not respond to imatinib mesylate [75].

Since Kit mutations of mast cell neoplasms and germ cell tumors were observed at the TK-II domain, imatinib mesylate may not be effective against these neoplasms [34, 75]. A remarkable effect of imatinib mesylate has not been reported in SCLC patients, either [76].

Imatinib mesylate is a derivative of 2-phenylaminopyrimidine. The effect of indolinone tyrosine kinase inhibitors has been tested in cell lines and dogs [77, 78]. Some of them inhibited tyrosine kinsae activity of Kit and showed the suppressive effect on a human SCLC cell line [77] and mast cell neoplasms of dogs [78]. Imatinib mesylate only inhibits the JM domain mutation of Kit, but indolinone derivatives inhibit both JM and TK-II domain mutations. There is a possibility that some indolinone derivatives might be effective against human mast cell neoplasms and germ cell tumors.

Conclusion

Loss-of-function mutations of Kit resulted in depletion of erythrocytes, melanocytes, germ cells, mast cells and ICCs. Although gain-of-function mutations of Kit were found in tumors of mast cells, germ cells and ICCs (GISTs), erythroleukemias and melanomas associated with the gain-of-function mutations of Kit have not been reported.

The significance of Kit gain-of-function mutations is most clearly identified in the case of GISTs, because an inhibitor of Kit, imatinib mesylate, shows a remarkable therapeutic effect on most GISTs [8, 75].

The Kit mutation appears to be an early event in development of GIST [51]. Although polyclonal hyperplasia of ICCs was observed throughout the GI tract of familial GIST patients with germline mutations of Kit [63] and knock-in mice of the mutant Kit gene [65], benign monoclonal GISTs develop within the hyperplasia. In addition to Kit mutations, other factors appear to be necessary for the development of even benign GISTs [8].

Although multiple benign GISTs develop in persons with germline mutation of Kit [58–62], most benign GISTs do not become malignant [8]. More than half of such persons with germline mutation of Kit survived to rather old age without development of malignant GISTs. This indicates that Kit mutation alone does not cause malignant transformation of GISTs. Kit is a good model for the biologi-

cal study of oncogenes as well as for studying the relationship between the location of tyrosine kinase mutations and the therapeutic effect of kinase inhibitors.

1 Russell E. S. (1979) Hereditary anemias of the mouse: a review for geneticists. Adv. Genet. **20:** 357–459
2 Chabot B., Stephenson D. A., Chapman V. M., Besmer P. and Bernstein A. (1988) The proto-oncogene c-kit encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. Nature **335:** 88–89
3 Geissler E. N., Ryan M. A. and Housman D. E. (1988) The dominant-white spotting (W) locus of the mouse encodes the c-kit proto-oncogene. Cell **55:** 185–192
4 Besmer P., Murphy J. E., George P. C., Qiu F., Bergold P. J., Lederman L. et al. (1986) A new acute transforming feline retrovirus and relationship of its oncogene v-kit with the protein kinase gene family. Nature **320:** 415–421
5 Furitsu T., Tsujimura T., Tono T., Ikeda H., Kitayama H., Koshimizu U. et al. (1993) 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. Invest. **92:** 1736–1744
6 Hirota S., Isozaki K., Moriyama Y., Hashimoto K., Nishida T., Ishiguro S. et al. (1998) Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science **279:** 577–580
7 Joensuu H., Roberts P. J., Sarlomo-Rikala M., Andersson L. C., Tervahartiala P., Tuveson D. et al. (2001) Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. N. Engl. J. Med. **344:** 1052–1056
8 Kitamura Y., Hirota S. and Nishida T. (2003) Gastrointestinal stromal tumors (GISTs): a model for molecule-based diagnosis and treatment of solid tumors. Cancer Sci. **94:** 315–320
9 Yarden Y., Kuang W. J., Yang-Feng T., Coussens L., Munemitsu S., Dull T. J. et al. (1987) Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. EMBO J. **6:**3341–3351.
10 Rosnet O., Marchetto S., deLapeyriere O. and Birnbaum D. (1991) Murine Flt3, a gene encoding a novel tyrosine kinase receptor of the PDGFR/CSF1R family. Oncogene **6:** 1641–1650
11 Nagata H., Worobec A. S., Oh C. K., Chowdhury B. A., Tannenbaum S., Suzuki Y. et al. (1995) Identification of a point mutation in the catalytic domain of the protooncogene c-kit in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. Proc. Natl. Acad. Sci. USA **92:** 10560–10564
12 Longley B. J., Tyrrell L., Lu S.-Z., Ma Y.-S., Langley K. and Ding T.-G. et al. (1996) Somatic c-kit activating mutation in urticaria pigmentosa and aggressive mastocytosis: establishment of clonality in a human mast cell neoplasm. Nat. Genet. **12:** 312–314
13 Tian Q., Frierson H. F. Jr, Krystal G. W. and Moskaluk C. A. (1999) Activating c-kit gene mutations in human germ cell tumors. Am. J. Pathol. **154:** 1643–1647
14 Nocka K., Tan J. C., Chiu E., Chu T. Y., Ray P., Traktman P. et al. (1990) Molecular bases of dominant negative and loss of function mutation at the murine c-kit/white spotting locus: W^{37}, W^{v}, W^{41} and W. EMBO J. **9:**1805–1813
15 Hayashi S., Kunisada T., Ogawa M., Yamaguchi K. and Nishikawa S. (1991) Exon skipping by mutation of an authentic splice site of c-kit gene in W/W mouse. Nucleic Acids Res. **19:** 1267–1271
16 Cable J., Barkway C. and Steel K. P. (1992) Characteristics of stria vascularis melanocytes of viable dominant spotting (W^{v}/W^{v}) mouse mutants. Hear. Res. **64:** 6–20
17 Kitamura Y., Go S. and Hatanaka K. (1978) Decrease of mast cells in W/W^{v} mice and their increase by bone marrow transplantation. Blood **52:** 447–452
18 Maeda H., Yamagata A., Nishikawa S., Yoshinaga K., Kobayashi S., Nishi K. et al. (1992) Requirement of c-kit for development of intestinal pacemaker system. Development **116:** 369–375
19 Tsujimura T., Hirora S., Nomura S., Niwa Y., Yamazaki M., Tono T. et al. (1991) Characterization of Ws mutant allele of rats: a 12-base deletin in tyrosine kinase domain of c-kit gene. Blood **78:** 1942–1946
20 Niwa Y., Kasugai T., Ohno K., Morimoto M., Yamazaki M., Dohmae K. et al. (1991) Anemia and mast cell depletion in mutant rats that are homozygous at white spotting (Ws) locus. Blood **78:** 1936–1941
21 Morimoto M., Kasugai T., Tei H., Jippo-Kanemoto T., Kanakura Y. and Kitamura Y. (1993) Age-dependent amelioration of hypoplastic anemia in Ws/Ws rats with a small deletion at the kinase domain of c-kit. Blood **82:** 3315–3320
22 Giebel L. B. and Spritz R. A. (1991) Mutation of the KIT (mast/stem cell growth factor receptor) protooncogene in human piebaldism. Proc. Natl. Acad. Sci. USA **88:** 8696–8699
23 Witte O. N. (1990) Steel locus defines new multipotent growth factor. Cell **63:** 5–6
24 Kitamura Y., Yokoyama M., Matsuda H. and Shimada M. (1980) Coincidental development of forestomach papilloma and prepyloric ulcer in nontreated mutant mice of W/W^{v} and Sl/Sl^{d} genotypes. Cancer Res. **40:** 3392–3397
25 Shimada M., Kitamura Y., Yokoyama M., Miyano Y., Maeyama K., Yamatodani A. et al. (1980) Spontaneous stomach ulcer in genetically mast cell depleted W/W^{v} mice. Nature **293:** 662–664
26 Kitamura Y. and Go S. (1979) Decreased production of mast cells in Sl/Sl^{d} anemic mice. Blood **53:** 492–497
27 Yokoyama M., Tatsuta M., Baba M., and Kitamura Y. (1982) Bile reflux: a possible cause of stomach ulcer in nontreated mutant mice of W/W^{v} genotype. Gastroenteology **82:** 857–863
28 Huizinga J. D., Thuneberg L., Kluppel M., Malysz J., Mikkelsen H. B. and Bernstein A. (1995) W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. Nature **373:** 347–349
29 Ward S. M., Burns A. J., Torihashi S., Harney S. C. and Sanders K. M. (1995) Impaired development of interstitial cells and intestinal electrical rhythmicity in steel mutants. Am. J. Physiol. **269:** 1577–1585
30 Thomsen L., Robinson T. L., Lee J. C., Farraway L. A., Hughes M. J., Andrews D. W. et al. (1998) Interstitial cells of Cajal generate a rhythmic pacemaker current. Nat. Med. **4:** 848–851
31 Isozaki K., Hirota S., Nakama A., Miyagawa J.-I., Shinomura Y., Xu Z. et al. (1995) Disturbed intestinal movement, bile reflux to the stomach and deficiency of c-kit-expressing cells in Ws/Ws mutant rats. Gastroenterology **109:** 456–464
32 Lemmon M. A., Pinchasi D., Zhou M., Lax I. and Schlessinger J. (1997) Kit receptor dimerization is driven by bivalent binding of stem cell factor. J. Biol. Chem. **272:** 6311–6317
33 Linnekin D. (1999) Early signaling pathways activated by c-Kit in hematopoietic cells. Int. J. Biochem. Cell Biol. **31:** 1053–1074
34 Chen H., Isozaki K., Kinoshita K., Ohashi A., Shinomura Y., Matsuzawa Y. et al. (2003) Imatinib inhibits various types of activating mutant KIT found in gastrointestinal stromal tumors. Int. J. Cancer **105:** 130–135
35 Foltz I. N. and Schrader J. W. (1997) Activation of the stress-activated protein kinases by multiple hematopoietic growth factors with the exception of interleukin-4. Blood **89:** 3092–3096
36 Zhang Y. Y., Vik T. A., Ryder J. W., Srour E. F., Jacks T., Shannon K. et al. (1998) Nf1 regulates hematopoietic progenitor cell growth and ras signaling in response to multiple cytokines. J. Exp. Med. **187:** 1893–1902
37 Weiler S. R., Mou S., DeBerry C. S., Keller J. R., Ruscetti F. W., Ferris D. K. et al. (1996) JAK2 is associated with the c-kit proto-oncogene product and is phosphorylated in response to stem cell factor. Blood **87:** 3688–3693

38 Linnekin D., DeBerry C. S. and Mou S. (1997) Lyn associates with the juxtamembrane region of c-Kit and is activated by stem cell factor in hematopoietic cell lines and normal progenitor cells. J. Biol. Chem. **272:** 27450–27455

39 Tsujimura T., Furitsu T., Morimoto M., Isozaki K., Nomura S., Matsuzawa Y. et al. (1994) 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

40 Tsujimura T., Furitsu T., Morimoto M., Kanayama Y., Nomura S., Matsuzawa Y. et al. (1995) 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

41 Wolfgang R. S., Horny H. S. and Valent P. (2002) Spectrum of associated clonal hematologic non-mast cell lineage disorder occurring in patients with systemic mastocytosis. Int. Arch. Allergy Immunol. **127:** 140–142

42 Longley B. J. Jr, Metcalfe D. D., Tharp M., Wang X., Tyrrell L. Lu S. Z. et al. (1999) Activating and dominant inactivating c-kit catalytic domain mutations in distinct forms of human mastocytosis. Proc. Natl. Acad. Sci. USA **96:** 1609–1614

43 Hartmann K., and Henz B. M. (2001) Mastocytosis: recent advances in defining the disease. Br. J. Dermatol. **144:** 682–695

44 London C. A., Galli S. J., Yuuki T., Hu Z. Q., Helfand S. C. and Geissler E. N. (1999) Spontaneous canine mast cell tumors express tandem duplications in the proto-oncogene c-kit. Exp. Hematol. **27:** 689–697

45 Nakao, M., Yokota, S., Iwai, T., Kaneko, H., Horiike, S., Kashima, K. et al. (1996) Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. Leukemia **10:** 1911–1918

46 Hashimoto K., Tsujimura T., Moriyama Y., Yamatodani A., Kimura M., Tohya K. et al. (1996) Transforming and differentiation-inducing potentials of constitutively activated c-kit mutant genes in the IC-2 murine interleukin-3-dependent mast cell line. Am. J. Pathol. **148:** 189–200

47 Koyasu S., Nakauchi H., Kitamura K., Yonehara S., Okumura K., Tada T. et al. (1985) Production of interleukin 3 and gamma-interferon by an antigen-specific mouse suppressor T cell clone. J. Immunol. **134:** 3130–3136

48 Kitayama H., Kanakura Y., Furitsu T., Tsujimura T., Oritani K., Ikeda H. et al. (1995) Consitutively activating mutantions of c-kit receptor tyrosine kinase confer factor-independent growth and tumorigenicity of factor-dependent hematopoietic cell lines. Blood **85:** 790–798

49 Tsujimura T., Morimoto M., Hashimoto K., Moriyama Y., Kitayama H., Matsuzawa Y. et al. (1996) 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

50 Kindblom L. G., Remotti H. E., Aldenborg F. and Meis-Kindblom J. M. (1998) Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am. J. Pathol. **152:** 1259–1269

51 Corless C. L., McGreevey L., Haley A., Town A. and Heinrich M. C. (2002) KIT mutations are common in incidental gastrointestinal stromal tumors one centimeter or less in size. Am. J. Pathol. **160:** 1567–1572

52 Lux M. L., Rubin B. P., Biase T. L., Chen C.-J., Maclure T., Demetri G. et al. (2000) KIT extracellular and kinase domain mutations in gastrointestinal stromal tumors. Am. J. Pathol. **156:** 791–795

53 Lasota J., Wozniak A., Sarlomo-Rikala M., Rys J., Kordek R., Nassar A. et al. (2000) Mutations in exons 9 and 13 of KIT gene are rare events in gastrointestinal stromal tumors. Am. J. Pathol. **157:** 1091–1095

54 Hirota S., Nishida T., Isozaki K., Taniguchi M., Nakamura J., Okazaki T. et al. (2001) Gain-of-function mutation at the extracellular domain of KIT in gastrointestinal stromal tumours. J. Pathol. **193:** 505–510

55 Rubin B. P., Singer S., Tsao C., Duensing A., Lux M. L., Ruiz R. et al. (2001) KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. Cancer Res. **61:** 8118–8121

56 Heinrich M. C., Corless C. L., Duensing A., McGreevey L., Chen C. J., Joseph N. et al. (2003) PDGFRA activating mutations in gastrointestinal stromal tumors. Science **299:** 708–710

57 Hirota S., Ohashi A., Nishida T., Isozaki K., Kinoshita K., Shinomura Y. et al. (2003) Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. Gastroenterology **125:** 660–667

58 Nishida T., Hirota S., Taniguchi M., Hashimoto K., Isozaki K., Nakamura H. et al. (1998) Familial gastrointestinal stromal tumours with germline mutation of the KIT gene. Nat. Genet. **19:** 323–324

59 Hirota S., Okazaki T., Kitamura Y., O'Brien P., Kapusta L. and Dardick I. (2000) Cause of familial and multiple gastrointestinal autonomic nerve tumors with hyperplasia of interstitial cells of Cajal is germline mutation of the c-kit gene. Am. J. Surg. Pathol. **24:** 326–327

60 Hirota S., Nishida T., Isozaki K., Taniguchi M., Nishikawa K., Ohashi A. et al. (2002) Familial gastrointestinal stromal tumors associated with dysphagia and novel type germline mutation of KIT gene. Gastroenterology **122:** 1493–1499

61 Isozaki K., Terris B., Belghiti J., Schiffmann S., Hirota S., Vanderwinden J.-M. (2000) Germline-activating mutation in the kinase domain of KIT gene in familial gastrointestinal stromal tumors. Am. J. Pathol. **157:** 1581–1585

62 Beghini A., Tibiletti M. G., Roversi G., Chiaravalli A. M., Serio G., Capella C. et al. (2001) Germline mutation in the juxtamembrane domain of the kit gene in a family with gastrointestinal stromal tumors and urticaria pigmentosa. Cancer **92:** 657–662

63 Chen H., Hirota S., Isozaki K., Sun H., Ohashi A., Kinoshita K. et al. (2002) Polyclonal nature of diffuse proliferation of interstitial cells of Cajal in patients with familial and multiple gastrointestinal stromal tumours. Gut **51:** 793–796

64 Maeyama H., Hidaka E., Ota H., Minami S., Kajiyama M., Kuraishi A. et al. (2001) Familial gastrointestinal stromal tumor with hyperpigmentation: association with a germline mutation of the c-kit gene. Gastroenterology **120:** 210–215

65 Sommer G, Agosti V., Ehlers I., Rossi F., Corbacioglu S, Farkas J. et al. (2003) Gastrointestinal stromal tumors in a mouse model by target mutation of the Kit receptor tyrosine kinase. Proc. Natl. Acad. Sci. USA **100:** 6706–6711

66 Sakuma Y., Sakurai S., Oguni S., Hironaka M. and Saito K. (2003) Alterations of the c-kit gene in testicular germ cell tumors. Cancer Sci. **94:** 486–491

67 Kemmer K., Corless C. L., Fletcher J .A., McGreevey L, Haley A., Griffith D. et al. (2004) KIT mutations are common in testicular seminomas. Am. J. Pathol. **164:** 305–313

68 Sekido Y., Obata Y., Ueda R., Hida T., Suyama M., Shinokata K. et al. (1991) Preferential expression of c-kit protooncogene transcripts in small cell lung cancer. Cancer Res. **51:** 2416–2419

69 Hibi K., Takahashi T., Sekido Y., Ueda R., Hida T., Ariyoshi Y. et al. (1991) Coexpression of stem cell factor and the c-kit genes in small-cell lung cancer. Oncogene **6:** 2291–2296

70 Mauro M. J. and Druker B. J. (2001) STI571: targeting BCR-ABL as therapy for CML. Oncologist **6:** 233–238

71 Druker B.J., Tamura S., Buchdunger E., Ohno S., Segal G.M., Fanning S., et al. (1996) Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat. Med. **2:** 561–566

72 Buchdunger E., Cioffi C. L., Law N., Stover D., Ohno-Jones S., Druker B. J. et al. (2000) Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. J. Pharmacol. Exp. Ther. **295:** 139–145

73 Tuveson D. A., Willis N. A., Jacks T., Griffin J. D., Singer S., Fletcher C. D. et al. (2001) STI571 inactivation of the gastrointestinal stromal tumor c-KIT oncoprotein: biological and clinical implications. Oncogene **20:** 5054–5058
74 Demetri G. D., von Mehren M., Blanke C. D., Van den Abbeele A. D., Eisenberg B., Roberts P. J. et al. (2002) Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N. Engl. J. Med. **347:** 472–480
75 Heinrich M. C., Corless C. L., Demetri G. D., Blanke C. D., von Mehren M., Joensuu H. et al. (2003) Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J. Clin. Oncol. **21:** 4342–4349
76 Soria J. C., Johnson B. E. and Chevalier T. L. (2003) Imatinib in small cell lung cancer. Lung Cancer **41 Suppl 1:** S49–S53
77 Abrams T. J., Lee L. B., Murray L. J., Pryer N. K. and Cherrington J. M. (2003) SU11248 inhibits KIT and platelet-derived gowth factor beta in preclinical models of human small cell lung cancer. Mol. Cancer Ther. **2:** 471–478
78 London C. A., Hannah A. L., Zadovoskaya R., Chien M. B., Kollias-Baker C. Rosenberg M. et al. (2003) Phase I dose-escalating study of SU11654, a small molecule receptor tyrosine kinsae inhibitor, in dogs with spontaneous malignancies. Clin. Cancer Res. **9:** 2755–2768