c-*kit* gene mutations in intracranial germinomas

Yuji Sakuma,[1] Shinji Sakurai,[1,3] Sachiko Oguni,[1] Masaaki Satoh,[2] Mitsugu Hironaka[1] and Ken Saito[1]

[1]Department of Pathology, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi-machi, Kawachi-gun, Tochigi 329-0498; [2]Department of Clinical Pathology, Sapporo Medical University Hospital, South 1 West 16, Chuo-ku, Sapporo 060-8543

(Received June 22, 2004/Revised July 20, 2004/Accepted July 26, 2004)

Gain-of-function mutations of the c-*kit* gene and the expression of phosphorylated KIT are found in most gastrointestinal stromal tumors and mastocytosis. Further, almost all gonadal seminomas/dysgerminomas exhibit KIT membranous staining, and several reports have clarified that some (10–25%) have a c-*kit* gene mutation. But, whether intracranial germinomas also have a c-*kit* gene mutation remains unsolved. To elucidate the presence, frequency, and location of c-*kit* gene mutations in intracranial germinomas, we analyzed five mutational hot spots (exons 9, 10, 11, 13, and 17) in the c-*kit* genomic DNA of 16 germinomas using polymerase chain reaction and direct sequencing. We found c-*kit* gene mutations at exon 11 (W557C) or 17 (D816V, D820V, and N822Y) in four germinomas (25.0%), although no statistically significant difference in any clinicopathological factor was found between patients with or without mutations. These results are similar to those seen in gonadal seminoma/dysgerminoma patients, and confirm that intracranial germinomas are exact counterparts of gonadal seminomas/dysgerminomas, as would be expected on histological and immunohistochemical grounds. Moreover, molecular targeting drugs such as imatinib mesylate (STI571), which is a selective inhibitor of KIT, might be promising agents for the treatment of intracranial germinomas with c-*kit* gene mutations. (Cancer Sci 2004; 95: 716–720)

The c-*kit* proto-oncogene encodes a receptor tyrosine kinase (KIT) that is a member of the same subfamily as the receptors for platelet-derived growth factor and colony-stimulating factor-1. KIT consists of an extracellular domain with five immunoglobulin-like repeats, a transmembrane domain, a juxtamembrane domain, and tyrosine kinase (TK) I and II domains split by a kinase insert. The ligand for KIT is stem cell factor (SCF). The SCF-KIT system is critical for the normal development and survival of melanocytes, erythrocytes, germ cells, mast cells, and interstitial cells of Cajal (ICCs).[1–3] On the other hand, gain-of-function mutations of the c-*kit* gene and the expression of phosphorylated KIT have been reported in tumors arising from these cell lineages, such as mast cell tumors,[4,5] gastrointestinal stromal tumors (GISTs),[6–13] which are thought to originate from ICCs in the gastrointestinal tract, and germ cell tumors (GCTs).[14–18] Four mutational hot spots in different regions of the c-*kit* gene have been identified: in exons 9, 11, 13, and 17. More recently, an additional activating c-*kit* gene mutation has been detected at exon 10 encoding the transmembrane domain.[19]

Imatinib mesylate (STI571; Gleevec; Novartis Pharma, Basel, Switzerland) is a selective inhibitor of certain tyrosine kinases including KIT, and has proven very effective in the treatment of patients with advanced GISTs, which are resistant to conventional chemotherapy, especially those harboring c-*kit* mutations at exon 11.[20] However, mutant KIT with Asp816Val at exon 17, which is the most common type of c-*kit* mutation in adult mastocytosis, is resistant to imatinib *in vitro*.[21] Thus, accurate evaluation of c-*kit* gene mutations seems to be indispensable for the effective use of imatinib.

In testicular GCTs, membranous KIT expression is characteristic of seminomas, but not non-seminomas.[22,23] Several reports, including a previous study by the authors, have shown the presence of c-*kit* gene mutations in GCTs. The most common type of mutation is an isolated point mutation at the TK II domain (exon 17), whereas we also found a point mutation at the juxtamembrane domain (exon 11).[14–18] To date, c-*kit* gene mutations have been reported in testicular seminomas,[14,16–18] an ovarian dysgerminoma,[14] and mediastinal seminomas.[15] However, only one report describes KIT expression in intracranial germinomas[24] and it is still unknown whether c-*kit* gene mutations are also present in intracranial germinomas. This study analyzed all mutational hot spots of the c-*kit* genomic DNA from biopsy specimens of 16 germinomas to clarify the frequency and location of c-*kit* gene mutations in intracranial germinomas.

Materials and Methods

Patients and tumor specimens. Sixteen formalin-fixed, paraffin-embedded biopsy specimens, histologically diagnosed as intracranial germinomas, were obtained from archives of the Department of Pathology at Jichi Medical School and the Department of Clinical Pathology at Sapporo Medical University Hospital. All tumor samples were reviewed by one of the authors (Y.S) to verify the diagnosis. The patients included 12 men and 4 women with a median age of 17 years (range, 9 to 30 years).

Immunohistochemical study. Immunohistochemical evaluations were performed using the avidin-biotin-peroxidase complex method with 3-μm-thick sections of the formalin-fixed, paraffin-embedded specimens. Polyclonal antibodies against human KIT (IBL, Fujioka, Japan) were used as the primary antibodies at working dilutions of 1:100. A monoclonal antibody against the Ki-67 antigen (MIB-1; MBL, Nagoya, Japan; 1:100 dilution) was used to assess the proportion of proliferating tumor cells. The Ki-67 labeling index (LI) was defined as the ratio of MIB-1-stained tumor cells to all tumor cells counted ×100. To evaluate the Ki-67 LI, numbers of stained tumor cells were counted in at least 3 high-power fields that showed the highest positivity for each section.

Genomic DNA extraction and sequencing. Genomic DNAs were extracted from 16 paraffin-embedded germinoma tissues using a standard proteinase K digestion method.[14] To amplify exons 9, 10, 11, 13, and 17 of the c-*kit* gene, the genomic DNAs were amplified by polymerase chain reaction. The primers for the amplification of exons 9, 10, 11, 13, and 17 are listed in Table 1. The amplified fragments were purified using a GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences, Piscataway, NJ) and direct sequencing was performed as described previously.[16]

Statistical analysis. Values are shown as the mean±SD. Statistical analysis was performed using Fisher's exact probability test, Student's *t* test and Mann-Whitney's *U* test, and values of $P<0.05$ were considered statistically significant.

Results

Table 2 summarizes the clinicopathological features, immuno-

[3]To whom correspondence should be addressed. E-mail: ssakurai@jichi.ac.jp

histochemistry, and c-*kit* gene mutations of the 16 intracranial germinoma patients studied.

Histology of intracranial germinomas. The tumors were composed of uniform cells resembling primordial germ cells, with large vesicular nuclei and a clear cytoplasm. They arranged themselves in a cobblestone pattern, and delicate fibrovascular septa variably infiltrated by small lymphocytes were usually observed (Fig. 1A). A strong similarity between intracranial germinomas and gonadal seminomas/dysgerminomas was confirmed.

Expression of KIT. Immunohistochemically, all 16 intracranial germinomas revealed membranous KIT expression in the majority of tumor cells (Fig. 1B).

Frequency and location of c-*kit* gene mutations in germinomas. Mutations were found in 4 of the 16 germinomas (25.0%). One germinoma contained a mutation at exon 11 encoding the juxtamembrane, while the others contained mutations at exon 17 encoding the TK II domain, as follows: Case 2 showed a point mutation at codon 557 (TGG to TGT) that resulted in a Trp557 to Cys (W557C) substitution at exon 11. In case 2, the wild-type sequence was almost undetectable. Thus, W557C might be a homozygous missense mutation (Fig. 2B). Cases 8, 9, and 13 showed a mutation at exon 17. Case 8 showed a point mutation at codon 822 (AAT to TAT) that resulted in an Asn822 to Tyr (N822Y) substitution (Fig. 3A), case 9 showed a point mutation at codon 820 (GAT to GTT) that resulted in an Asp820 to Val (D820V) substitution (Fig. 3B), and case 13 showed a point mutation at codon 816 (GAC to GTC) that resulted in an Asp816 to Val (D816V) substitution.

Moreover, two different sequence alterations were observed

Table 1. Summary of primer sequences

Name	Sequence
PCR amplification and sequencing of c-*kit* exons 9, 10, 11, 13, and 17	
Exon 9F	5′-ATGCTCTGCTTCTGTACTGCC-3′
Exon 9R	5′-CAGAGCCTAAACATCCCCTTA-3′
Exon 10F	5′-ATTGTAGAGCAAATCCATCCCC-3′
Exon 10R	5′-GCCCCTGTTTCATACTGACCA-3′
Exon 11F	5′-CCAGAGTGCTCTAATGACTG-3′
Exon 11R	5′-ACCCAAAAAGGTGACATGGA-3′
Exon 13F	5′-CATCAGTTTGCCAGTTGTGC-3′
Exon 13R	5′-ACACGGCTTTACCTCCAAATG-3′
Exon 17F	5′-TGTATTCACAGAGACTTGGC-3′
Exon 17R	5′-GGATTTACATTATGAAAGTCACAGG-3′

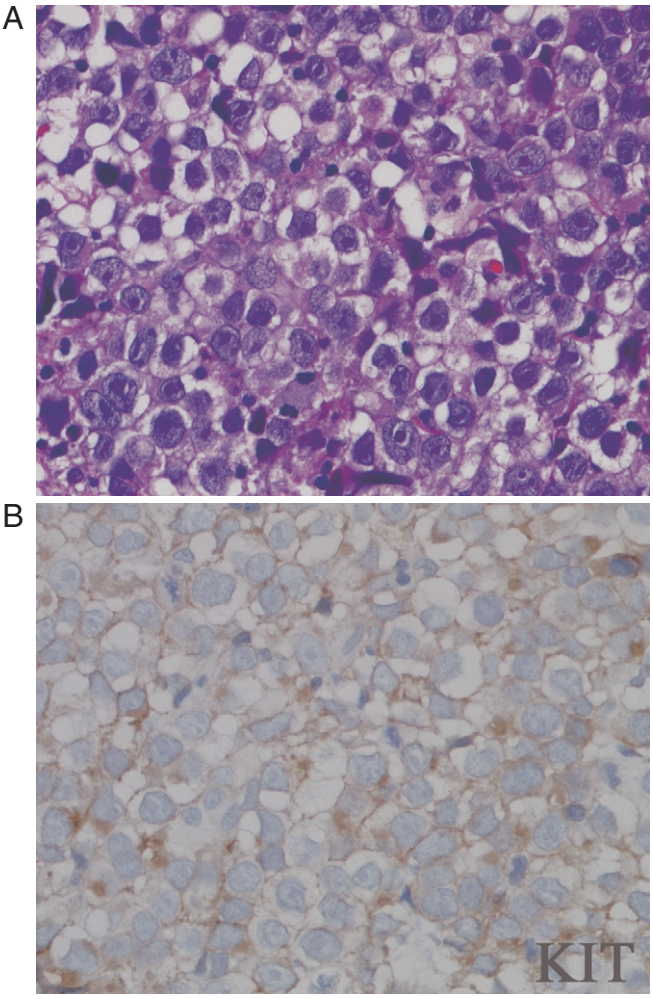

Fig. 1. Histology and KIT immunohistochemical staining of case 13. (A) The histology of intracranial germinomas is similar to that of testicular seminomas. (B) Germinomas showing uniform membranous KIT staining in the tumor cells.

Table 2. Clinicopathological data, immunohistochemistry and c-*kit* gene mutations of 16 intracranial germinoma patients

Case No.	Age	Sex	Primary site	Maximum size (cm)	Ki-67 LI (%)	IHC KIT	c-*kit* gene mutation	Follow-up
1	13	F	neurohypophyseal	N.A	48.0	+	wild	AW, 167 mos
2	19	M	fourth ventricle	N.A	58.6	+	W557C	N.A
3	21	M	pineal	2.0	61.2	+	wild	AW, 73 mos
4	25	M	lateral ventricle	N.A	58.3	+	wild	AW, 59 mos
5	10	M	pineal	3.0	48.5	+	wild	AW, 12 mos
6	9	M	neurohypophyseal	2.0	58.8	+	wild	AW, 94 mos
7	30	F	neurohypophyseal	3.0	47.4	+	wild	AW, 25 mos
8	17	M	neurohypophyseal	1.0	58.2	+	N822Y	DOD, 53 mos
9	10	M	basal ganglia	N.A	56.6	+	D820V	AW, 74 mos
10	13	M	pineal	3.0	56.3	+	wild	AW, 62 mos
11	13	M	neurohypophyseal	4.0	58.4	+	wild	AW, 64 mos
12	13	M	pineal	2.5	N.A[1)]	+	wild	AW, 57 mos
13	20	M	basal ganglia	3.0	56.9	+	D816V	AW, 35 mos
14	17	F	neurohypophyseal	N.A	45.9	+	wild	AW, 55 mos
15	21	M	pineal	N.A	74.8	+	wild	AW, 21 mos
16	24	F	neurohypophyseal	3.0	N.A[1)]	+	wild	AW, 3 mos

LI, labeling index; IHC, immunohistochemistry; N.A, not available; +, positive; AW, alive and well; DOD, died of disease; mos, months.
1) Available tumor tissues were too small to allow evaluation of the Ki-67 labeling index.

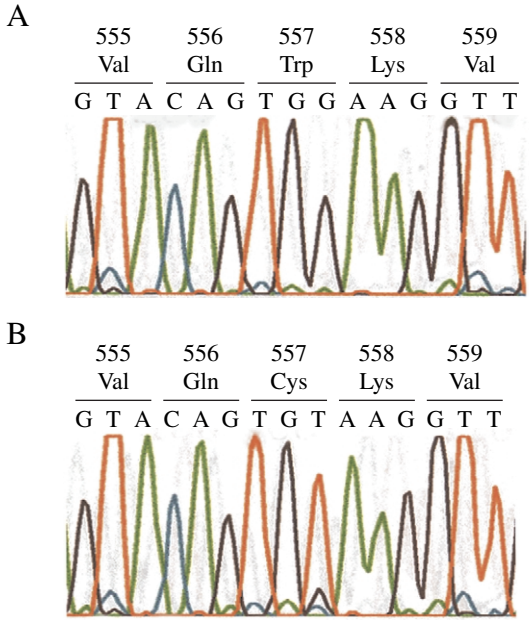

Fig. 2. Genomic sequencing of the c-*kit* gene at exon 11. (A) Case 1 showing a wild-type sequence. (B) Case 2 showing a point mutation at codon 557 (TGG to TGT). Trp557 is changed to Cys. Since the wild-type sequence of position 3 at codon 557 is almost undetectable, this mutation might be a homozygous mutation.

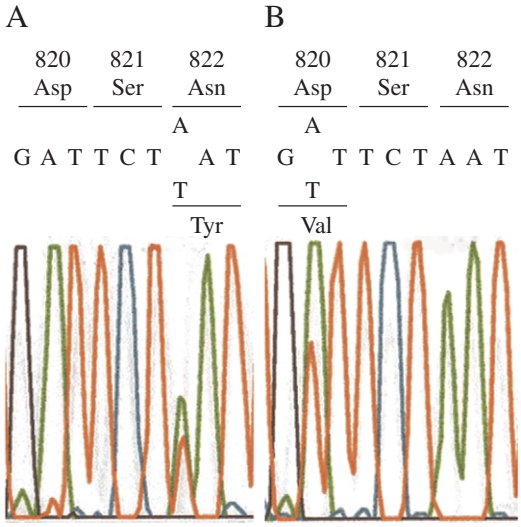

Fig. 3. Genomic sequencing of the c-*kit* gene at exon 17. (A) Case 8 showing a point mutation at codon 822. Asn822 is changed to Tyr. (B) Case 9 demonstrating a point mutation at codon 820. Asp820 is changed to Val.

at exon 10. One was observed at codon 541 (ATG to CTG), resulting in a Met541 to Leu (M541L) substitution in case 11, and the other was detected at codon 546 (AAA to AAG), resulting in no amino acid substitution (Lys546 to Lys, K546K) in case 7. According to the dbSNP Home Page (http://www.ncbi.nlm.nih.gov/SNP/), the M541L substitution is a single nucleotide polymorphism. An identical sequence alteration at codon 546 (AAA to AAG) was observed in both the neoplastic and non-neoplastic tissues of a patient with a pulmonary carcinoma in our study (data not shown), indicating that the alteration of AAA to AAG at codon 546 detected in this study is also a polymorphism.

Comparison of germinoma patients with and without c-*kit* gene mutations. Clinicopathological data were compared between germinoma patients with and without c-*kit* gene mutations. No statistically significant differences were found between the two groups (Table 3).

Table 3. Comparison of clinicopathological data between germinoma patients with and without c-*kit* gene mutations

	Mutation (+) (n=4)	Mutation (−) (n=12)	Statistics
Age (median)	18 (10–20)	15 (9–30)	NS
Sex (male:female)	4:0	8:4	NS
Maximum size (cm)	2.0±1.4 (n=2)	2.8±0.7 (n=8)	NS
Ki-67 LI (%)	57.6±1.0 (n=4)	55.8±8.8 (n=10)	NS
Alive and well	2/3 (n=3)	12/12 (n=12)	NS

NS, not significant; LI, labeling index.

Discussion

In this study, we confirmed the membranous KIT expression of tumor cells in all germinomas examined. This result is similar to that in the case of testicular seminomas.[16, 22, 23] Moreover, we revealed the presence of c-*kit* gene mutations in 4 of the 16 (25.0%) intracranial germinomas. This frequency is higher than that of testicular GCTs (4 of 34, 11.8%) in our previous report,[16] but almost equal to that reported by Kemmer *et al*.,[18] who found activating point mutations at exon 17 in 24.1% (13 of 54) of the pure testicular seminomas studied. No germinomas had a mutation in more than one locus. Three out of the four germinomas with mutations had a point mutation at exon 17 (D816V, D820V, and N822Y). The D816V mutation has been shown to cause constitutive phosphorylation of KIT *in vitro*, and in *de novo* human mastocytosis[4, 5] and testicular seminomas.[18] Point mutations at codon 820 have been detected in aggressive mastocytosis (D820G),[25] familial GISTs (D820Y),[10] and a mediastinal seminoma (D820V).[15] A minority of GISTs (N822K, N822H)[11] and testicular seminomas (N822K)[18] have a point mutation at codon 822. Thus, the three mutations identified at exon 17 in the present study are considered to be a gain-of-function type. The other c-*kit* gene mutation was detected at exon 11 (W557C). Codon 557 is often affected in sporadic GISTs.[11] In addition, a point mutation at codon 557 has also been found in familial GISTs[26] and a testicular seminoma.[16] Therefore, the W557C mutation detected in this study is also thought to be a gain-of-function type. However, no apparent differences in clinicopathological factors were observed between patients with and without c-*kit* gene mutations.

Previously, we reported that a subset of testicular GCTs (4 of 34 cases, 11.8%) contained a somatic point mutation at exon 11 (W557R, n=1) or 17 (D816V, n=2; D816H, n=1).[16] In gonadal seminomas/dysgerminomas, although the most common type of c-*kit* gene mutation has been found at codon 816 (D816V or D816H),[14, 16–18] codons 557 (W557R), 822 (N822K), and 823 (Y823D and Y823C) have also been affected.[16, 18] Moreover, in mediastinal seminomas, missense point mutations at exon 17 (K818R, D820V, and N822K) have been observed.[15] Thus, this study shows that the location of c-*kit* gene mutations in intracranial germinomas agrees with those in gonadal and mediastinal seminomas/dysgerminomas. Our present results, such as frequency and location of the c-*kit* gene mutation, and membranous KIT expression, confirm that germinomas are the intracranial counterparts of gonadal seminomas/dysgerminomas, as would be histologically expected.

To date, activating c-*kit* gene mutations have been identified in about 90% of mastocytosis[5] and GISTs.[11] The c-*kit* gene mutations in mastocytosis predominantly involve exon 17, which encodes the TK II domain, and this is especially true for the D816V point mutation. In contrast, mutations in GISTs mainly involve exon 11, which encodes the juxtamembrane domain. These data indicate that activating c-*kit* gene mutations have a crucial role in the pathogenesis of these tumors. On the other hand, c-*kit* gene mutations have been found in only 10–25% of intracranial germinomas and sporadic testicular seminomas.[16, 18] These results lead us to surmise that c-*kit* gene mutations do not play a critical role in the development of germinomas/seminomas. In testicular seminomas, phosphorylated KIT was detectable only in seminomas with c-*kit* gene mutations, but not ones without the mutations.[18] Thus, KIT expression might be a "differentiation marker" in intracranial germinomas without c-*kit* gene mutations, as well as testicular seminomas without the mutations, whereas in germinomas/seminomas with c-*kit* gene mutations, mutant KIT might play a role in the development or maintenance of the tumors.[18] The testicular seminomas previously examined by the authors[16] and Kemmer *et al*.[18] were sporadic tumors and most were thought to be unilateral tumors, because the incidence of bilateral testicular GCTs is quite rare and was reported to be only 1–2% in testicular GCT patients.[27, 28] As established by the seminal study of Looijenga *et al*.,[17] almost all bilateral testicular GCTs contain a somatic activating point mutation at codon 816 of the c-*kit* gene (57 of 61, 93.4%). Moreover, the types of mutations in the bilateral GCTs were identical in each patient. These results indicated that c-*kit* gene mutations might occur in primordial germ cells during or before migration to the genital ridge, and play a role in their tumorigenesis. Thus, they concluded that c-*kit* gene mutations were associated with the development of bilateral testicular GCTs, while the detection of mutations in unilateral tumors identified patients at risk of bilateral disease. On the other hand, about 2.5% (16 of 635) of patients with extragonadal (retroperitoneal or mediastinal) GCTs reportedly develop testicular GCTs, with a median time between diagnoses of 60 months.[29] There is also a report of metachronous testicular seminoma in a patient with pineal germinoma.[30] This raises the possibility that identical c-*kit* gene mutations are present in both extragonadal and gonadal GCTs, although mutational analyses in such cases have yet to be conducted. From these results, we speculate that somatic c-*kit* gene mutations are not essential for, but do promote, the development of some seminomas/germinomas. In this study, 4 patients with intracranial germinomas with c-*kit* gene mutations might be at risk for other gonadal or extragonadal GCTs, although second GCTs had not been diagnosed at 35 to 74 months after the first diagnosis.

The newly developed molecular targeting drug imatinib is an ATP-competitive tyrosine kinase inhibitor for c-ABL, BCR/ABL, platelet-derived growth factor receptor, and KIT. Imatinib inhibits wild-type and juxtamembrane domain (exon 11) mutant KIT *in vitro*.[21] Clinically significant responses to imatinib have been reported in advanced GISTs with c-*kit* gene mutations, which resist conventional chemotherapy.[20] However, the application of imatinib to intracranial germinomas has yet to be tried. Although mutant KIT with a codon 816 mutation at exon 17 is resistant to imatinib inhibition, mutant KIT with a codon 822 or 823 mutation is sensitive to imatinib in GISTs.[31] It is very important to recognize that the locus of c-*kit* gene mutations affects sensitivity to imanitib. Thus, the germinomas with W557C, D820V, and N822Y mutations of the c-*kit* gene detected in this study might be sensitive to imatinib. Moreover, PP1, which was recently identified as an inhibitor of Src tyrosine kinase families, has been reported to inhibit the activation of mutant KIT with a point mutation at codon 816, as well as wild-type KIT.[32] Thus, PP1 might be effective for GCTs and mastocytosis with a point mutation at codon 816.

Typically, intracranial germinomas are highly radiosensitive, and a high cure rate with radiation alone or cisplatin-base chemotherapy followed by low-dose radiotherapy has been achieved. However, several late adverse effects, such as secondary malignancy and cognitive endocrinological and visual dysfunctions, have been observed in some patients with germinomas.[33] Thus, molecular targeting drugs, such as imatinib or PP1, could be adopted as another choice of therapy for patients with germinomas.

In conclusion, we have found the presence of mutations at exons 11 and 17 of the c-*kit* gene in 25.0% (4 of 16) of the intracranial germinomas studied. All mutations identified in this study were considered gain-of-function types, although, as with testicular GCTs, there were no statistically significant differences in any clinicopathological factors between patients with and without c-*kit* gene mutations. Since germinoma patients with c-*kit* gene mutations might be at risk for secondary GCTs, such patients should be followed up for several years. Molecular targeting drugs such as imatinib or PP1 might be effective against intracranial germinomas with c-*kit* gene mutations. Further studies are needed to understand the role of KIT expression and c-*kit* gene mutations in the development of gonadal, extragonadal, and intracranial germ cell tumors.

1. Kitamura Y, Hirota S, Nishida T. A loss-of-function mutation of c-*kit* results in depletion of mast cells and interstitial cells of Cajal, while its gain-of-function mutation results in their oncogenesis. *Mutat Res* 2001; **477**: 165–71.
2. Ashman LK. The biology of stem cell factor and its receptor C-*kit*. *Int J Biochem Cell Biol* 1999; **31**: 1037–51.
3. Rossi P, Sette C, Dolci S, Geremia R. Role of c-*kit* in mammalian spermatogenesis. *J Endocrinol Invest* 2000; **23**: 609–15.
4. Furitsu T, Tsujimura T, Tono T, Ikeda H, Kitayama H, Koshimizu U, Sugahara H, Butterfield JH, Ashman LK, Kanayama Y, MatsuzawaY, Kitamura Y, Kanakura 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 Invest* 1993; **92**: 1736–44.
5. Longley BJ Jr, Metcalfe DD, Tharp M, Wang X, Tyrrell L, Lu SZ, Heitjan D, Ma Y. Activating and dominant inactivating c-*KIT* catalytic domain mutations in distinct clinical forms of human mastocytosis. *Proc Natl Acad Sci USA* 1999; **96**: 1609–14.
6. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Tunio GM, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y. Gain-of-function mutations of c-*kit* in human gastrointestinal stromal tumors. *Science* 1998; **279**: 577–80.
7. Lux ML, Rubin BP, Biase TL, Chen CJ, Maclure T, Demetri G, Xiao S, Singer S, Fletcher CDM, Fletcher JA. KIT extracellular and kinase domain mutations in gastrointestinal stromal tumors. *Am J Pathol* 2000; **156**: 791–5.
8. Isozaki K, Terris B, Belghiti J, Schiffmann S, Hirota S, Vanderwinden JM. Germline-activating mutation in the kinase domain of *KIT* gene in familial gastrointestinal stromal tumors. *Am J Pathol* 2000; **157**: 1581–5.
9. Hirota S, Nishida T, Isozaki K, Taniguchi M, Nakamura J, Okazaki T, Kitamura Y. Gain-of-function mutation at the extracellular domain of KIT in gastrointestinal stromal tumours. *J Pathol* 2001; **193**: 505–10.
10. Hirota S, Nishida T, Isozaki K, Taniguchi M, Nishikawa K, Ohashi A, Takabayashi A, Obayashi T, Okuno T, Kinoshita K, Chen H, Shinomura Y, Kitamura Y. Familial gastrointestinal stromal tumors associated with dysphagia and novel type germline mutation of KIT gene. *Gastroenterology* 2002; **122**: 1493–9.
11. Rubin BP, Singer S, Tsao C, Duensing A, Lux ML, Ruiz R, Hibbard MK, Chen CJ, Xiao S, Tuveson DA, Dementri GD, Fletcher CDM, Fletcher JA. KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res* 2001; **61**: 8118–21.
12. Sakurai S, Oguni S, Hironaka M, Fukayama M, Morinaga S, Saito K. Mutations in c-*kit* gene exon 9 and 13 in gastrointestinal stromal tumors among Japanese. *Jpn J Cancer Res* 2001; **92**: 494–8.
13. Sakurai S, Fukasawa T, Chong JM, Tanaka A, Fukayama M. C-*kit* gene abnormalities in gastrointestinal stromal tumors (tumors of interstitial cells of Cajal). *Jpn J Cancer Res* 1999; **90**: 1321–8.
14. Tian Q, Frierson HF Jr, Krystal GW, Moskaluk CA. Activating c-*kit* gene mutations in human germ cell tumors. *Am J Pathol* 1999; **154**: 1643–7.

15. Przygodzki RM, Hubbs AE, Zhao FQ, O'Leary TJ. Primary mediastinal seminomas: evidence of single and multiple *KIT* mutations. *Lab Invest* 2002; **82**: 1369–75.
16. Sakuma Y, Sakurai S, Oguni S, Hironaka M, Saito K. Alterations of the c-*kit* gene in testicular germ cell tumors. *Cancer Sci* 2003; **94**: 486–91.
17. Looijenga LH, de Leeuw H, van Oorschot M, van Gurp RJ, Stoop H, Gillis AJ, de Gouveia Brazao CA, Weber RF, Kirkels WJ, van Dijk T, von Lindern M, Valk P, Lajos G, Olah E, Nesland JM, Fossa SD, Oosterhuis JW. Stem cell factor receptor (c-KIT) codon 816 mutations predict development of bilateral testicular germ-cell tumors. *Cancer Res* 2003; **63**: 7674–8.
18. Kemmer K, Corless CL, Fletcher JA, McGreevey L, Haley A, Griffith D, Cummings OW, Wait C, Town A, Heinrich MC. KIT mutations are common in testicular seminomas. *Am J Pathol* 2004; **164**: 305–13.
19. Akin C, Fumo G, Yavuz AS, Lipsky PE, Neckers L, Metcalfe DD. A novel form of mastocytosis associated with a transmembrane c-kit mutation and response to imatinib. *Blood* 2004; **103**: 3222–5.
20. Demetri GD, von Mehren M, Blanke CD, Van Den Abbeele AD, Eisenberg B, Roberts PJ, Heinrich MC, Tuveson DA, Singer S, Janicek M, Fletcher JA, Silverman SG, Silverman SL, Capdeville R, Kiese B, Peng B, Dimitrijevic S, Druker BJ, Corless C, Fletcher CDM, Joensuu H. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med* 2002; **347**: 472–80.
21. Ma Y, Zeng S, Metcalfe DD, Akin C, Dimitrijevic S, Butterfield JH, McMahon G, Longley BJ. The c-*KIT* mutation causing human mastocytosis is resistant to STI571 and other KIT kinase inhibitors; kinases with enzymatic site mutations show different inhibitor sensitivity profiles than wild-type kinases and those with regulatory-type mutations. *Blood* 2002; **99**: 1741–4.
22. Izquierdo MA, Van der Valk P, Van Ark-Otte J, Rubio G, Germa-Lluch JR, Ueda R, Scheper RJ, Takahashi T, Gianccone G. Differential expression of the c-*kit* proto-oncogene in germ cell tumours. *J Pathol* 1995; **177**: 253–8.
23. Strohmeyer T, Reese D, Press M, Ackermann R, Hartmann M, Slamon D. Expression of the c-*kit* proto-oncogene and its ligand stem cell factor (SCF) in normal and malignant human testicular tissue. *J Urol* 1995; **153**: 511–5.
24. Miyanohara O, Takeshima H, Kaji M, Hirano H, Sawamura Y, Kochi M, Kuratsu J. Diagnostic significance of soluble c-kit in the cerebrospinal fluid of patients with germ cell tumors. *J Neurosurg* 2002; **97**: 177–83.
25. Pignon JM, Giraudier S, Duquesnoy P, Jonault H, Imbert M, Vainchenker W, Vernant JP, Tulliez M. A new c-kit mutation in a case of aggressive mast cell disease. *Br J Haematol* 1997; **96**: 374–6.
26. Hirota S, Okazaki T, Kitamura Y, O'Brien P, Kapusta L, Dardick I. 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* 2000; **24**: 326–7.
27. Che M, Tamboli P, Ro JY, Park DS, Ro JS, Amato RJ, Ayala AG. Bilateral testicular germ cell tumors: twenty-year experience at M.D. Anderson Cancer Center. *Cancer* 2002; **95**: 1228–33.
28. Theodore Ch, Terrier-Lacombe MJ, Laplanche A, Benoit G, Fizazi K, Stamerra O, Wibault P. Bilateral germ-cell tumours: 22-year experience at the Institut Gustave Roussy. *Br J Cancer* 2004; **90**: 55–9.
29. Hartmann JT, Fossa SD, Nichols CR, Droz JP, Horwich A, Gerl A, Beyer J, Pont J, Fizazi K, Hecker H, Kanz L, Einhorn L, Bokemeyer C. Incidence of metachronous testicular cancer in patients with extragonadal germ cell tumors. *J Natl Cancer Inst* 2001; **93**: 1733–8.
30. Peat DS, Trowell JE. Testicular seminoma in a patient with pineal germinoma. *J Clin Pathol* 1994; **47**: 771–2.
31. Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, McGreevey LS, Chen CJ, Van den Abbeele AD, Druker BJ, Kiese B, Eisenberg B, Roberts PJ, Singer S, Fletcher CD, Silberman S, Dimitrijevic S, Fletcher JA. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol* 2003; **21**: 4342–9.
32. Tatton L, Morley GM, Chopra R, Khwaja A. The Src-selective kinase inhibitor PP1 also inhibits Kit and Bcr-Abl tyrosine kinases. *J Biol Chem* 2003; **278**: 4847–53.
33. Kaur H, Singh D, Peereboom DM. Primary central nervous system germ cell tumors. *Curr Treat Options Oncol* 2003; **4**: 491–8.