Evidence for the involvement of a hematopoietic progenitor cell in systemic mastocytosis from single-cell analysis of mutations in the c-*kit* gene

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Mast cells are derived from multipotential hematopoietic progenitors and are clonally increased in systemic mastocytosis, a disease associated with point mutations of codon 816 (most commonly Asp816Val) of c-*kit*. To study the lineage relationship and the extent of expansion of cells derived from the mutated clone, we examined the occurrence of the Asp816Val c-*kit* mutation in genomic DNA of individual sorted peripheral blood T cells, B cells, and monocytes in patients with indolent systemic mastocytosis. The mutation was detected in varying frequencies in the genomic DNA of individual B cells and monocytes and bone marrow mast cells in patients with extensive disease. In B cells, the immunoglobulin repertoire was polyclonal, indicating that the mutation occurred before $V_H/(D)/J_H$ recombination. These results show that mastocytosis is a disorder of a pluripotential hematopoietic progenitor cell that gives rise to B cells and monocytes in addition to mast cells and that the affected clone shows variable expansion in these lineages in the peripheral blood of patients with systemic mastocytosis. (Blood. 2002; 100:661-665)

Introduction

Mastocytosis is a rare disorder characterized by a pathologic increase in mast cells in tissues including skin, bone marrow, liver, spleen, and lymph nodes. Activating mutations in c-*kit*, the gene encoding the receptor for Kit ligand, also known as stem cell factor, may be detected in the bone marrow, peripheral blood, and lesional skin in patients with mastocytosis.[1,2] These mutations most commonly involve codon 816 of the intracellular tyrosine kinase domain and cause ligand-independent autophosphorylation of the receptor. An additional mutation in codon 560 within the juxtamembrane domain of Kit, yielding a substitution of glycine for valine, has been detected in the human mast cell leukemia line HMC-1[3] and, in one study, in some patients with mastocytosis.[4]

Recently, messenger RNA (mRNA) encoding the Asp816Val c-*kit* mutation has been detected in non–mast cell hematopoietic lineages in the peripheral blood and bone marrow in patients with indolent mastocytosis and in those with an associated hematologic disorder.[5] This observation suggests that in mastocytosis, the mutation in c-*kit* occurs in a pluripotential hematopoietic progenitor cell. Lineage relationship and the frequency of the cells derived from the progenitor with the mutated c-*kit*, however, could not be established because the observations relied on the analysis of bulk mRNA.

The current study was therefore carried out to determine the lineage relationship of cells derived from the hematopoietic progenitor with the c-*kit* mutation, to assess whether the cells derived from the mutated clone coexist with those carrying the wild-type gene, and to document the involvement of a hematopoietic progenitor cell in disease pathogenesis. To achieve these goals, we examined the occurrence of the Asp816Val and the Val560Gly c-*kit* mutations in the genomic DNA of individual peripheral blood T cells, B cells, and monocytes in 5 patients with indolent systemic mastocytosis using single-cell mutational analysis. The Asp816Val c-*kit* mutation was detected in varying frequencies in the genomic DNA of B cells and monocytes in 3 patients with extensive mastocytosis. These results demonstrate that mastocytosis is a clonal disorder of a pluripotential hematopoietic progenitor cell and that the affected clone shows variable expansion in these cell lineages in the peripheral blood of patients with systemic mastocytosis.

Patients, materials, and methods

Patients

Five patients with adult-onset indolent systemic mastocytosis were studied after informed consent according to the Declaration of Helsinki was obtained on a protocol approved by the institutional review board of the National Institute of Allergy and Infectious Diseases (Table 1). Patients had elevated plasma levels of surrogate disease markers, tryptase[6] and soluble CD117.[7] Three patients (no. 3, 4, and 5; Table 1) had hypercellular bone marrows (> 70% cellularity with myeloid hyperplasia but no overt clonal hematologic non–mast cell lineage disorder) in addition to markedly elevated plasma levels of surrogate markers. The clinical and laboratory features of these patients met the criteria to be classified in the "smoldering mastocytosis" subcategory of indolent systemic mastocytosis.[8]

Cell sorting

Peripheral blood mononuclear cells were isolated using Ficoll-Hypaque density gradient centrifugation. $CD3^+$ T cells, $CD19^+$ B cells, and $CD14^+$ monocytes were stained with lineage-specific monoclonal antibodies (BD Pharmingen, San Diego, CA). Each cell lineage was first sorted in bulk

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Table 1. Patient demographics

Patient	Category	M/F	Age, y	Duration of disease, y	Tryptase, ng/mL	sCD117, AU/mL	Bone marrow
1	ISM	F	39	13	26	480	NC, focal
2	ISM	F	49	9	139	753	NC, focal
3	SSM	M	45	30	164	1865	HC, diffuse
4	SSM	M	71	33	217	4755	HC, focal
5	SSM	M	67	9	262	ND	HC, focal

M indicates male; F, female; sCD117, soluble CD117 (soluble Kit); AU, arbitrary units; ISM, indolent systemic mastocytosis; NC, normocellular; SSM, smoldering systemic mastocytosis; HC, hypercellular; and ND, not determined.

using the FACStar flow cytometer (Becton Dickinson, San Diego, CA). After this procedure, single cells were sorted by flow cytometry into individual wells of 96-well polymerase chain reaction (PCR) plates (USA Scientific, Bethesda, MD) containing 20 μL 1 times PCR buffer (Promega, Madison, WI) and proteinase-K (0.2 mg/mL; Sigma, St Louis, MO). The purity of each sorted population was more than 99% as determined by flow cytometry.

Mast cells were isolated from a bone marrow aspirate from patient 3 and bone marrow biopsy tissue from patient 4. Bone marrow aspirate mononuclear cells from patient 3 were incubated with CD117-phycoerythrin (PE) and CD25–fluorescein isothiocyanate (FITC) at 4°C for 30 minutes, and mast cells were sorted into individual wells as $CD117^+$, $CD25^+$ cells with high side-scatter. Bone marrow biopsy tissue from patient 4 was incubated in phosphate-buffered saline (PBS) containing 2 mM EDTA and 0.1% bovine serum albumin (BSA) for 2 hours. It was then disaggregated mechanically by cutting the tissue into small pieces and passing it through a 19-gauge needle several times followed by filtering through a cell strainer. The cell suspension prepared from the bone marrow biopsy tissue was incubated with an anti-CD25 antibody conjugated to PE (BD Pharmingen) at 4°C for 30 minutes, washed once with PBS supplemented with 0.1% BSA, and incubated with anti-PE paramagnetic microbeads (Miltenyi Biotec, Auburn, CA). Cells expressing surface CD25 were then isolated according to manufacturer's instructions. Cytospin preparations obtained from $CD25^+$ cells and stained with Wright-Giemsa stain following this procedure showed mast cells and lymphoid cells, as expected. Mast cells were then distinguished from lymphoid cells based on their characteristic high side-scatter pattern and were sorted into the single wells of a 96-well PCR plate with a FACStar flow cytometer.

Single-cell PCR and sequencing

Cells sorted into 96-well PCR plates were incubated for 1 hour at 55°C and 10 minutes at 95°C. Genomic DNA was then preamplified using random 15-mer primers and 60 rounds of amplification with *Taq* polymerase, as described.[9] The preamplified genomic DNA was then subjected to nested PCR (Table 2). Both juxtamembrane and tyrosine kinase domains of c-*kit* were amplified, sequenced, and analyzed for the presence of Gly560Val and Val816Asp mutations, respectively. The first region (c-*kit*–R1) containing codon 560 spans exon 10, intron 10, and exon 11. The second region (c-*kit*–R2) spans exon 17 and intron 17 and contains codon 816.

To assess the presence of mutations, PCR products were purified with QIAquick PCR purification kit (Qiagen, Hilden, Germany) and directly sequenced using an ABI Prism 377 automated DNA sequencer (Applied Biosystems, Foster City, CA) and a Big Dye Terminator DNA sequencing kit (Applied Biosystems, Foster City, CA). The sequences were aligned with the GenBank sequence of human c-*kit* (accession number, U63834) using the software Lasergene (DNASTAR, Madison, WI).

The PCR error rate in this study was calculated by analyzing 75 182 base pairs (bp) of c-kit sequence obtained from 257 individual cells (79 B cells, 86 monocytes, 92 mast cells) from patient 4. A total of 6 nucleotide errors were detected, indicating that the PCR error rate was 8×10^{-5}/nucleotide.

Immunoglobulin gene $V_H/(D)/J_H$ rearrangements from individual B cells were amplified and sequenced as described.[9]

To determine the frequency of the 81517C>T polymorphism of c-*kit*, bulk genomic DNA was isolated from the peripheral blood mononuclear cells of 22 patients with mastocytosis and 69 healthy donors. Genomic DNA was then subjected to nested PCR using c-*kit* R2 primers and the conditions shown in Table 2. PCR products were purified, sequenced, and analyzed as described above. A peak consisting of C and T nucleotides in equal heights were observed in individuals carrying the polymorphism as a heterozygous allele. A single peak of either C or T was observed in patients carrying homozygous wild-type or polymorphic alleles.

Results

Detection of the Asp816Val c-*kit* mutation in individual sorted mast cells, monocytes, and B cells

Initially, a 292-bp tyrosine kinase fragment spanning exon 17 and intron 17 of the c-*kit* genomic DNA was examined in single-sorted T cells, B cells, monocytes, and mast cells from patients with mastocytosis. Of 1824 cells sorted, 1292 (71%) yielded sequences suitable for analysis. In peripheral blood of patients 3, 4, and 5, the Asp816Val c-*kit* mutation was detectable in varying frequencies ranging from 3.8% to 89.5% of the $CD14^+$ monocytes and 10.3% to 55.7% of the $CD19^+$ B cells (Table 3). The frequency of the monocytes and B cells carrying the mutation was lower in patient 3,

Table 2. C-*kit* primers used in this study

Primer	Sequence	Annealing temp, °C	$MgCl_2$ concentration, mM	Length, bp
External				
c-*kit*–R1	5′-GCTGTGGTAGAGATCCCAT-3′	50	2	469
	5′-CGCAATTTCACAGAAAACTC-3′			
c-*kit*–R2	5′-TATTCACAGAGACTTGGCAG-3′	50	2.5	420
	5′-ATTTCTCCTGCTGTGACCTT-3′			
Nested				
c-*kit*–R1	5′-ATTGTAGAGCAAATCCATCCCC-3′	60	2	308
	5′-GCCCCTGTTTCATACTGACCA-3′			
c-*kit*–R2	5′-CCTCCTTACTCATGGTCGGATC-3′	60	2.5	292
	5′-CTGTTTCCTTCACATGCCCC-3′			

Table 3. Frequency of Asp816Val c-*kit* mutation in peripheral blood of patients with mastocytosis

	T lymphocytes			B lymphocytes			Monocytes		
Patient	No. cells analyzed	Cells with mutation	% positive	No. cells analyzed	Cells with mutation	% positive	No. cells analyzed	Cells with mutation	% positive
1	82	0	0	87	0	0	69	0	0
2	34	0	0	14	0	0	27	0	0
3	68	0	0	78	8	10.3	78	3	3.8
4	88	0	0	79	44	55.7	86	77	89.5
5	60	1	1.7	80	21	26.3	65	29	44.6

who also had a lower serum tryptase level when compared with patients 4 and 5. The c-*kit* mutation was either absent or detectable in low frequency (in 1 of 60 cells from patient 5) in T cells. In addition, the mutation was detectable in 29 of 110 (26.4%) and 158 of 187 (84.5%) sequences obtained from bone marrow mast cells of patients 3 and 4, respectively.

The single-cell analysis made it possible to amplify and sequence immunoglobulin heavy-chain genes amplified from the same individual B cells carrying the Asp816Val mutations from patient 4. The sequences obtained (n = 23) were polyclonal with no evidence of monoclonal expansion (data not shown). These results indicate that the Asp816Val mutation in c-kit occurred before V/(D)/J recombination, prior to the pro–B-cell stage of B-cell differentiation.

The Asp816Val mutation was not detectable in any of the cell lineages examined in patients 1 and 2. Patients 3, 4, and 5, who had mutations detectable in multiple cell lineages, had disease that was classified as systemic smoldering mastocytosis as characterized by a high degree of mast cell infiltration of the bone marrow, myeloid hyperplasia, and higher plasma levels of tryptase.[8]

Codon 560 mutations are not present in cells with the Asp816Val mutation or wild-type c-*kit*

Because the HMC-1 cell line derived from a patient with mast cell leukemia contains a codon 560 c-*kit* mutation in addition to the Asp816Val mutation[3] and because there is one report of detection of this mutation in patients with mastocytosis,[4] we next examined the juxtamembrane codon 560 in exon 10 for mutations in the same DNA samples. We evaluated a total of 468 sequences from patients 1 through 4 including 95 sequences from bone marrow mast cells of patient 4. No mutations were detected in codon 560 in any of the sequences examined.

Detection and frequency of a novel polymorphism in c-*kit* gene

Analysis of the sequencing results of the PCR products revealed the presence of 2 c-*kit* polymorphisms. The first of these polymorphisms resulted in a Met→Leu substitution (A75544C) in one patient (no. 3). The frequency of this polymorphism has been reported to be similar in patients with mastocytosis and healthy subjects.[10]

In addition, a novel polymorphism (81517C>T) in intron 17 of the c-*kit* gene was identified. The 81517C>T c-*kit* polymorphism was detected in 857 of 1054 (81.3%) amplifications from 4 patients (patients 2-5) bearing this polymorphism in a heterozygous state. Because of the apparent high frequency of this polymorphism in our study population, we determined whether this polymorphism was present in increased frequency in patients with mastocytosis as compared to healthy individuals. We thus analyzed genomic DNA from 22 patients with mastocytosis and 69 healthy control subjects. The allelic frequency of this polymorphism was found to be similar in patients with mastocytosis compared to the healthy population (36% versus 31%, respectively). We therefore concluded that 81517C>T c-*kit* polymorphism is not significantly associated with mastocytosis.

The single-cell technique used in this study made it possible to determine whether the mutation and the polymorphism occurred on the same or different alleles. This was analyzed in patient 4, who had a high frequency of mast cells with the Asp816Val mutation (Figure 1). In many mast cells (149 of 187, 80%) both c-*kit* alleles were amplified and the relation between the mutation and the polymorphism could not be determined (Figure 1B,C). However, in other cells (38 of 187, 20%), only one allele was amplified (Figure 1A,D). This made it possible to determine whether the mutation and the polymorphism occurred on the same allele. Notably, the mutation and the polymorphism never (0 of 38) occurred in the same sequence. Thus, 12 sequences contained the mutation but not the polymorphism (Figure 1A), whereas 26 sequences had the polymorphism but not the mutation (Figure 1D). These data strongly imply that the mutation and the polymorphism were found exclusively on different alleles.

Discussion

The single-cell genomic DNA analysis presented in this study is the first clear documentation of the involvement of a multipotential hematopoietic progenitor cell in systemic mastocytosis. As a result of a mutation in a common hematopoietic progenitor, the mutation could be detected in mast cells, monocytes, and B cells in variable frequencies in 3 patients with extensive disease (Table 1).

The cell lineages found to have the Asp816Val mutation in this study correlate with the reported occurrence of this mutation in bulk mRNA samples obtained from these hematopoietic populations in patients with mastocytosis.[5] Thus, detection of the mutation in peripheral blood B cells and monocytes and bone marrow mast cells may be explained by the mutational targeting of c-*kit* in a progenitor cell with the restricted capacity to differentiate into these lineages. Alternatively, the mutation may occur in a more primitive precursor cell, but it may provide a preferential growth advantage only to B cells, monocytes, and mast cells, although the mutation does not result in accumulation of excessive numbers of B cells and monocytes in the peripheral blood. It is noteworthy, however, that patients with the c-*kit* mutation detectable in B cells and monocytes did have hepatosplenomegaly and hypercellular bone marrow, indicating that they may have had an increased total body burden of hematopoietic cells. It should also be noted that the c-*kit* mutation was found in one T cell. Whether this represents a PCR error or indicates that the *c-kit* mutation also occurs in T cells at a low frequency cannot be determined. However, the data do indicate that the frequency of the Asp816Val mutation in c-*kit* is much lower in T cells than in B cells and monocytes.

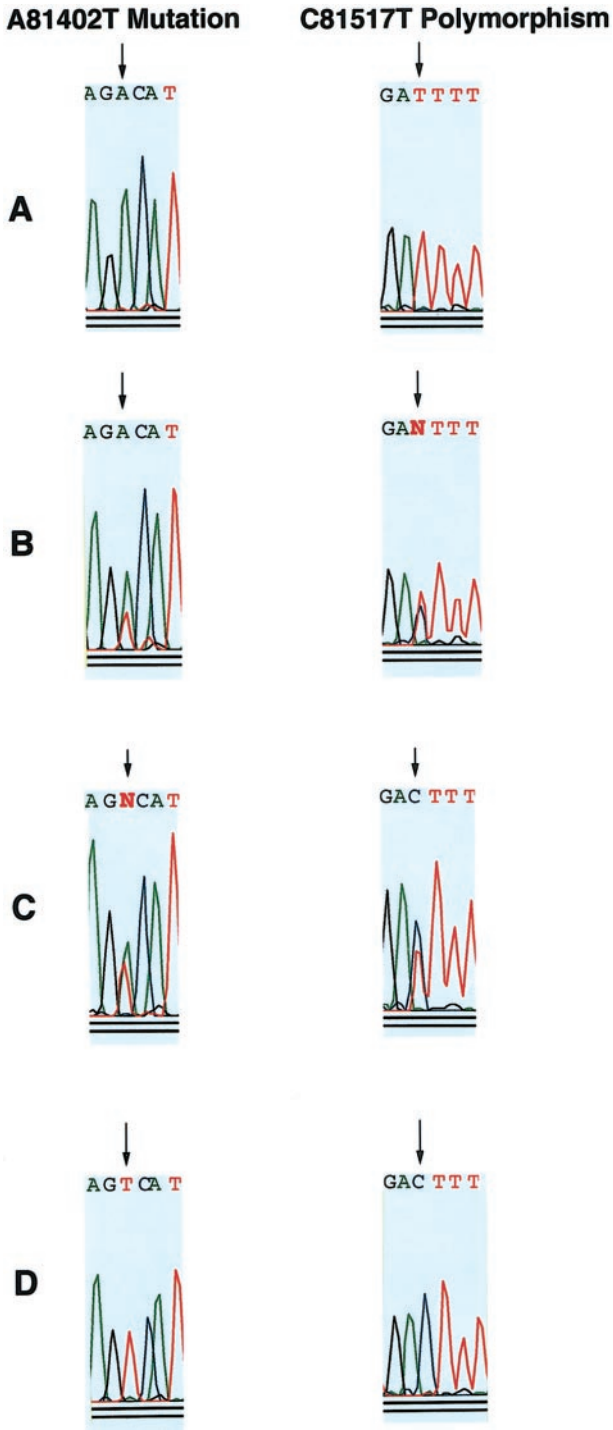

Figure 1. Demonstration of the A81402T (Asp816Val) mutation and C81517T (81517C>T) polymorphism of c-*kit* in 187 individual mast cells by single-cell PCR amplification of genomic DNA followed by sequencing. The regions containing the mutation and the polymorphism in the same electropherogram from 4 individual bone marrow mast cells (A-D) are shown. Panel A is typical of 12 of 187 cells in which the mutation but not the polymorphism was detected. Panels B and C are typical of 149 of 187 cells in which both the polymorphism and the mutation were detected. Panel D is typical of 26 cells in which the polymorphism but not the mutation was detected.

An analogous lineage involvement pattern has been reported in chronic myeloid leukemia (CML) and in myelodysplastic syndromes (MDSs). Thus, combined use of fluorescence-activated cell sorting and fluorescence in situ hybridization revealed the presence of the Philadelphia chromosome in myeloid cells, most B cells, and bone marrow T-cell progenitors, but not in mature peripheral T cells in the chronic phase of CML.[11] Similar observations were reported in patients with MDS.[12,13] These findings are consistent with the conclusion that systemic mastocytosis, similar to CML and MDS, is a clonal disorder of a hematopoietic progenitor cell with a capacity to give rise to B cells and myeloid cells preferentially. The demonstration of a polyclonal rearrangement pattern for the immunoglobulin heavy chain in B cells carrying the mutation is also consistent with this conclusion.

Our data strongly support the concept that systemic mastocytosis, at least the smoldering subvariant, is closely related to the classical group of chronic myeloproliferative diseases. These diseases are characterized by the clonal involvement of a hematopoietic progenitor cell leading to excessive numbers of mature cells belonging to one or more of the myeloid lineages. The other common features shared by systemic smoldering mastocytosis and chronic myeloproliferative diseases include the frequent presence of hepatosplenomegaly, increased bone marrow cellularity, and the activation of tyrosine kinase signal transduction pathways.

All 3 of the patients who had demonstrable multilineage involvement in this study met the criteria to be included in the recently established "indolent smoldering mastocytosis" category.[14-16] This category defines patients with extensive mast cell burden as evidenced by more than 30% infiltration of the bone marrow cavity by mast cells, elevated serum tryptase levels of more than 200 ng/mL, and hepatosplenomegaly. The expansion and the relative frequency of cells clonally derived from the hematopoietic progenitor carrying the c-*kit* mutation may thus correlate with the extent of the disease. The observation that the frequency of B cells and monocytes carrying the *c-kit* mutation was higher in patients with higher tryptase levels supports this explanation. A modest growth advantage conveyed by the c-*kit* mutation could account for the slowly developing expansion of affected hematopoietic lineages.

Our results did not identify the juxtamembrane Val560Gly c-*kit* mutation in cells carrying the Asp816Val c-*kit* mutation including lesional bone marrow mast cells. Consistent with this observation, juxtamembrane c-*kit* mutations generally are described to be associated with gastrointestinal stromal tumors but not mastocytosis.[17]

We have identified a novel polymorphism (81517C>T) in intron 17 of c-*kit*. This polymorphism was present in high frequency in both patients with mastocytosis and in healthy individuals. The occurrence of this polymorphism in a heterozygous state also served as an internal control for the sensitivity of the single cell PCR analysis. This nucleotide change was detectable in 857 of 1054 (81.3%) single-cell PCR products in patients carrying the polymorphism as opposed to the expected frequency of 100% as a germline polymorphism. This is likely to be a result of differential amplification of the heterozygous alleles during the initial nonspecific preamplification and the subsequent nested PCR steps. If the frequency data for c-*kit* Asp816Val is normalized according to an allelic dropout rate of 18.7%, 100% of the mast cells and monocytes and 68.5% of B cells analyzed in patient 4 can be estimated to carry the mutation.

Although the frequency of the 81517C>T polymorphism was not greater in patients with mastocytosis than in healthy blood donors, the amplification of c-*kit* genes from individual cells made it possible to demonstrate a potentially unique feature of the role of this polymorphism in mastocytosis. The analysis of c-*kit* gene in mast cells of patient 4 was particularly informative because nearly

all (84.5%) of these contained the Asp816Val mutation. The single-cell PCR technique usually (80%) amplifies both alleles from each cell. Most of the mast cells were clearly heterozygous for the mutation. It was notable, however, that examination of the height of the nucleotide peaks generated by the automated sequencer suggested that there was an inverse correlation between the height of the mutation peak and that of the polymorphism (Figure 1A,B). This suggested that the mutation and the polymorphism resided on different alleles. More compelling was the analysis of the 20% of the cells in which only one allele was amplified. In these cells, there was a complete discordance between the detection of the mutation and the polymorphism, indicating that they occurred on separate alleles. Analysis of the pattern of mutation and polymorphism in patients 3 and 5 revealed a similar pattern. Thus, in 53 mast cells from patient 3 and 75 monocytes from patient 5, the polymorphism and the mutation were never in the same sequence. This clearly documents the precision of the single-cell PCR technique, but also suggests that the development of mastocytosis may require 2 lesions of the *c-kit* gene. The activating Asp816Val mutation may be required in one allele along with the 81517C>T polymorphism that may serve to alter the expression or function of the other c-*kit* allele. Analysis of additional patients will be necessary to confirm or reject this hypothesis.

In conclusion, these data provide support for inclusion of systemic mastocytosis in the group of hematopoietic clonal disorders and offer insight into the nature of the progenitor cell affected by the c-*kit* mutation.

References

1. Nagata H, Worobec AS, Oh CK, et al. 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 U S A. 1995;92:10560-10564.
2. Longley BJ, Jr., Metcalfe DD, Tharp M, et al. Activating and dominant inactivating c-KIT catalytic domain mutations in distinct clinical forms of human mastocytosis. Proc Natl Acad Sci U S A. 1999;96:1609-1614.
3. Furitsu T, Tsujimura T, Tono T, et al. 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;92:1736-1744.
4. Buttner C, Henz BM, Welker P, Sepp NT, Grabbe J. Identification of activating c-kit mutations in adult-, but not in childhood-onset indolent mastocytosis: a possible explanation for divergent clinical behavior. J Invest Dermatol. 1998;111:1227-1231.
5. Akin C, Kirshenbaum AS, Semere T, et al. Analysis of the surface expression of c-kit and occurrence of the c-kit Asp816Val activating mutation in T cells, B cells, and myelomonocytic cells in patients with mastocytosis. Exp Hematol. 2000;28:140-147.
6. Schwartz LB, Sakai K, Bradford TR, et al. The alpha form of human tryptase is the predominant type present in blood at baseline in normal subjects and is elevated in those with systemic mastocytosis. J Clin Invest. 1995;96:2702-2710.
7. Akin C, Schwartz LB, Kitoh T, et al. Soluble stem cell factor receptor (CD117) and IL-2 receptor alpha chain (CD25) levels in the plasma of patients with mastocytosis: relationships to disease severity and bone marrow pathology. Blood. 2000;96:1267-1273.
8. Valent P, Horny HP, Li CY, et al. Mastocytosis. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2001:291-302.
9. Brezinschek HP, Brezinschek RI, Lipsky PE. Analysis of the heavy chain repertoire of human peripheral B cells using single-cell polymerase chain reaction. J Immunol. 1995;155:190-202.
10. Nagata H, Worobec AS, Metcalfe DD. Identification of a polymorphism in the transmembrane domain of the protooncogene c-kit in healthy subjects. Exp Clin Immunogenet. 1996;13:210-214.
11. Takahashi N, Miura I, Saitoh K, Miura AB. Lineage involvement of stem cells bearing the Philadelphia chromosome in chronic myeloid leukemia in the chronic phase as shown by a combination of fluorescence-activated cell sorting and fluorescence in situ hybridization. Blood. 1998;92:4758-4763.
12. Nilsson L, Astrand-Grundstrom I, Arvidsson I, et al. Isolation and characterization of hematopoietic progenitor/stem cells in 5q-deleted myelodysplastic syndromes: evidence for involvement at the hematopoietic stem cell level. Blood. 2000;96:2012-2021.
13. White NJ, Nacheva E, Asimakopoulos FA, et al. Deletion of chromosome 20q in myelodysplasia can occur in a multipotent precursor of both myeloid cells and B cells. Blood. 1994;83:2809-2816.
14. Valent P, Horny H, Escribano L, et al. Diagnostic criteria and classification of mastocytosis: a consensus proposal. Leuk Res. 2001;25:603-625.
15. Akin C, Scott LM, Metcalfe DD. Slowly progressive systemic mastocytosis with high mast-cell burden and no evidence of a non-mast-cell hematologic disorder: an example of a smoldering case? Leuk Res. 2001;25:635-638.
16. Jordan J, Fritsche-Polanz R, Sperr WR, et al. A case of "smouldering" mastocytosis with high mast cell burden, monoclonal myeloid cells, and C-KIT mutation Asp-816-Val. Leuk Res. 2001;25:627-634.
17. Hirota S, Isozaki K, Moriyama Y, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science. 1998;279:577-580.