Alterations of the c-*kit* gene in testicular germ cell tumors

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Expression and gain-of-function mutation of the c-*kit* gene, that encodes a receptor tyrosine kinase (KIT), have been reported in mast cell tumors and gastrointestinal stromal tumors (GISTs). Among human testicular germ cell tumors (GCTs), seminomas and seminoma components of mixed GCTs have also been shown to express KIT, but only one study has found the c-*kit* gene mutation at exon 17 in seminoma. To elucidate the frequency and location of the c-*kit* gene mutation of testicular GCTs, we analyzed the whole coding region of the c-*kit* complementary DNA along with 4 mutational hot spots (exons 9, 11, 13 and 17) of the c-*kit* genomic DNA by polymerase chain reaction and direct sequencing. Somatic mutations were found in 4 pure seminomas of 34 testicular GCTs (11.8%). One mutation was found in exon 11 (W557R) and the others were observed in exon 17 (D816H and D816V). These types of mutations were reported in GISTs (W557R), seminoma (D816H) and mastocytosis (D816V) and were considered to be gain-of-function mutations, although there were no differences of any clinicopathological factors or outcome between patients with and without mutations. Additionally, we also demonstrated coexpression of Gly-Asn-Asn-Lys$^{510-513}$ (GNNK)+ and GNNK− isoforms of the c-*kit* gene with dominance of the GNNK− transcript in all testicular GCTs. The mutations and/or preferential expression of GNNK− isoform of the c-*kit* gene might play an important role in the development of testicular GCTs, and these tumors may also be targets for STI571, which is a promising drug for advanced and metastatic GISTs. (Cancer Sci 2003; 94: 486–491)

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) 1 and 2 domains split by the kinase insert. The natural ligand for KIT is stem cell factor (SCF). Critical downstream signaling mechanisms include activation of cell survival (anti-apoptotic) proteins, such as AKT, and cell proliferation-related proteins, such as mitogen-activated protein kinase (p42/p44).

In normal tissues, the SCF-KIT system plays a crucial role in the development of melanocytes, erythrocytes, mast cells, interstitial cells of Cajal and germ cells.[1–3] Recently, expression of KIT and gain-of-function mutations of the c-*kit* gene have been identified in tumors arising from these cell lineages, such as human mast cell tumors,[4,5] gastrointestinal stromal tumors (GISTs)[6–11] and germ cell tumors (GCTs).[12] Moreover, coexpression of SCF and KIT has been demonstrated in other malignancies, such as small cell lung cancers[13] and Ewing's sarcomas.[14] In these tumors, an autocrine stimulation loop may play a role in tumor development.

To date, mutational hot spots in four different regions of the c-*kit* gene have been found. In sporadic and familial GISTs, mutations in exons 9, 11, 13 and 17 have been reported.[6–11,15,16] In mast cell tumors, mutations in exons 11 and 17 have been identified.[4,5] Recently, significant efficacy of STI571 (imatinib mesylate, Gleevec; Novartis Pharmaceuticals, East Hanover, NJ), which is a selective inhibitor of certain receptor tyrosine kinases including KIT, has been reported for advanced unresectable or metastatic GISTs that resist conventional chemotherapy.[17] Moreover, the presence or absence, and location of the c-*kit* gene mutations have been reported to influence the sensitivity of tumors to STI571.[18,19] Thus, an accurate evaluation of c-*kit* gene mutations seems to be indispensable for the use of STI571.

In testicular GCTs, KIT membranous expression is characteristic of most seminomas, but not non-seminomas,[20–22] and the alterations of KIT may play a central role in their pathogenesis. However, only a single study has evaluated testicular, ovarian and extragonadal GCTs for the presence of mutations in exons 11 and 17 of the c-*kit* gene by genomic DNA sequencing.[12] Mutations in exons 9 and 13 of the c-*kit* gene are also involved in pathogenesis of GISTs,[7–11] but mutations in these regions have not been reported in testicular GCTs.

To elucidate the frequency and location of the c-*kit* gene mutations in testicular GCTs, especially in seminomas and seminoma components of mixed GCTs, we analyzed the whole coding region of the c-*kit* complementary DNAs (cDNAs) from fresh frozen samples, and all mutational hot spots of the c-*kit* genomic DNAs from paraffin-embedded tissues. Additionally, we also analyzed the expression of SCF and KIT isoforms, which are also thought to be involved in the pathogenesis of some KIT-expressing tumors.

Materials and Methods

The present study protocol was approved by the ethical committee at Jichi Medical School.

Patients and tissue specimens. Thirty-four testicular GCTs were surgically resected from 34 patients. Of these, fresh frozen and formalin-fixed, paraffin-embedded materials were obtained from 14 tumors, while only formalin-fixed materials were available from 20 tumors. The age range of patients was 21 to 60 years (median 35 years). Histologically, tumors from 29 patients were classified as pure seminomas and the other 5 were classified as mixed GCTs including seminoma components. Histology of the mixed GCTs was as follows: case 1 was composed of teratoma, embryonal carcinoma, choriocarcinoma and seminoma components, case 2 of teratoma, embryonal carcinoma and seminoma components, and case 4 of embryonal carcinoma, yolk sac tumor and seminoma components. Both cases 7 and 13 were composed of embryonal carcinoma and seminoma components.

Immunohistochemical study. Immunohistochemical evaluations were performed using the avidin-biotin-peroxidase complex method in 3-μm-thick sections of formalin-fixed, paraffin-embedded specimens of testicular GCTs. Polyclonal antibodies against human KIT (MBL, Nagoya) and SCF (K089, IBL, Fujioka) were used as primary antibodies, at working dilutions

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of 1:100 and 1:50, respectively. Monoclonal antibody against Ki-67 antigen (MIB-1, MBL; 1:100 dilution) was used to assess the proportion of proliferating tumor cells. Ki-67 labeling index (LI) was defined as the ratio of MIB-1-stained tumor cells to all tumor cells counted ×100. Polyclonal antibody against single-stranded DNA (ssDNA, DAKO JAPAN, Kyoto; 1:100 dilution) was used to elucidate the proportion of the apoptotic tumor cells. Apoptotic index was defined as the ratio of ssDNA-stained tumor cells to all tumor cells counted ×100. Numbers of stained tumor cells were counted in at least 5 high-power fields that showed the highest positivity in each section for evaluation of the Ki-67 LI and the apoptotic index. In mixed GCTs, Ki-67 LI and apoptotic index were counted in seminoma components that were positive for KIT.

Sequencing analyses of the c-*kit* gene. Sequencing analyses of testicular GCTs were performed using cDNAs of the whole coding region and/or genomic DNAs of exons 9, 11, 13 and 17 of the c-*kit* gene. In the cases having c-*kit* gene mutation(s), genomic DNAs were isolated from paraffin sections of non-neoplastic companion tissues in the same sections.

cDNA sequencing: Total RNAs were prepared from 14 fresh-frozen tissues (9 seminomas and 5 mixed GCTs including seminoma components) with TRIZOL Reagent (GIBCO BRL, Rockville, MD) according to the manufacturer's recommendations. One microgram of total RNAs from fresh frozen tissues was reverse-transcribed to cDNA using a First-strand cDNA Synthesis Kit (Amersham Pharmacia Biotech, Tokyo). Then, to amplify the whole coding region of the c-*kit* gene, cDNAs were amplified by polymerase chain reaction (PCR) using the primers listed in Table 1. Each of the amplified fragments was purified from the polyacrylamide gel and direct sequencing was carried out using a Thermo Sequenase II Dye Terminator Cycle Sequencing Premix Kit (Amersham Pharmacia Biotech) and an ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster City, CA) with the same primers as used for PCR. All c-*kit* gene sequencing reactions were performed in both forward and reverse directions.

Genomic DNA sequencing: Genomic DNAs were extracted from the 34 formalin-fixed, paraffin-embedded tumor tissues, using a standard proteinase K digestion method. Then, the genomic DNAs were amplified by PCR using the primers listed in Table 1 to amplify exons 9, 11, 13 and 17 of the c-*kit* gene. Then, the amplified fragments were purified from polyacrylamide gels and direct sequencing was performed as described above.

RT-PCR analyses of GNNK+/– isoforms of c-*kit* and SCF. cDNAs from 14 fresh frozen tissues were amplified by PCR using the primers listed in Table 1 to evaluate expressions of the Gly-Asn-Asn-Lys$^{510-513}$ (GNNK)+/– isoforms of the c-*kit* gene and the soluble and membrane-bound SCF isoforms. cDNA from human testis was used as the control.

Statistical analysis. Values are shown as mean±SD. Statistical analysis was performed using Mann-Whitney's *U* test, and values of $P<0.05$ were considered significant.

Results

Table 2 summarizes the clinicopathological features, immunohistochemistry and c-*kit* gene mutations of the 34 testicular GCT patients studied.

Expressions of KIT and SCF proteins. Immunohistochemically, all

Table 1. Summary of primer sequences

Name	Sequence
PCR amplification and sequencing of KIT whole coding region	
PCRKIT 13s	5′-TCGCAGCTACCGCGATGAGA-3′
PCRKIT 14as	5′-TCACTTCTGGGTCTGTGAGA-3′
PCRKIT 15s	5′-CAGACCCAGAAGTGACCAATTA-3′
PCRKIT 16as	5′-CTCTCGCTGAACTGATAGTCAAC-3′
PCRKIT 17s	5′-GTTGACTATCAGTTCAGCGAGAG-3′
PCRKIT 18as	5′-ATTCACGAGCCTGTCGTAA-3′
PCRKIT 19s	5′-GCACTTACACATTCCTAGTGTCC-3′
PCRKIT 20as	5′-ACATCATGCCAGCTACGAT-3′
PCRKIT 21s	5′-ACTCCTTTGCTGATTGGTTTCGT-3′
PCRKIT 22s	5′-AATGGTGCAGGCTCCAAGTAGAT-3′
PCRKIT 23s	5′-CATGAATATTGTGAATCTACTTG-3′
PCRKIT 24as	5′-TGATCCGACCATGAGTAA-3′
PCRKIT 25s	5′-GACGAGTTGGCCCTAGAC-3′
PCRKIT 26as	5′-AGTTGGAGTAAATATGATTGGTG-3′
PCRKIT 27s	5′-TGCTGAAATGTATGACATAATGA-3′
PCRKIT 28as	5′-GGTAGAAGCTACGTTGCTATTG-3′
PCR amplification and sequencing of KIT exons 9, 11, 13 and 17	
Exon 9F	5′-ATGCTCTGCTTCTGTACTGCC-3′
Exon 9R	5′-CAGAGCCTAAACATCCCCTTA-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′
PCR amplification of KIT GNNK+/– isoform	
PCRKIT 3s	5′-GGGGGATCCGATGTGGGCAAGACTTCT-3′
PCRKIT 4as	5′-CAGCAAAGGAGTGAACAG-3′
PCR amplification of SCF	
SCF s	5′-ATTTTCAATAGATCCATTGA-3′
SCF as	5′-CCAGTATAAGGCTCCAAAAGCAA-3′

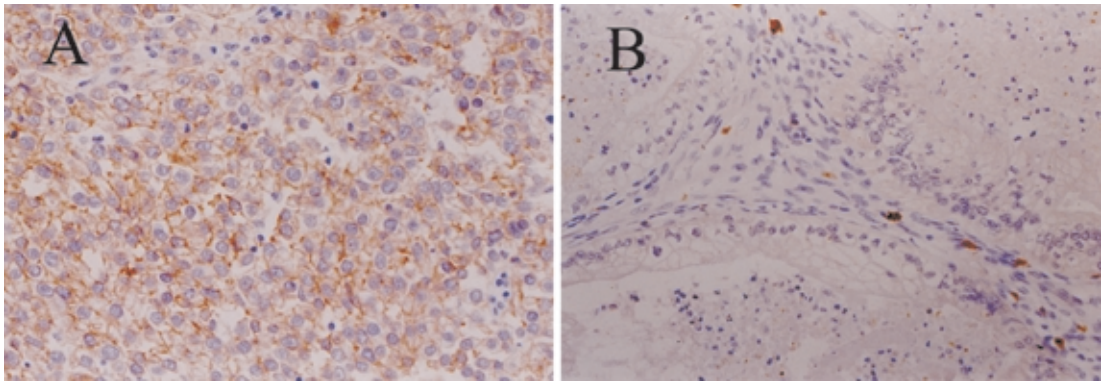

Fig. 1. KIT immunohistochemical staining of case1 (mixed GCT). (A) The seminoma component reveals uniform membranous staining of tumor cells. (B) Tumor cells in the teratoma component do not express KIT, whereas the admixed mast cells express KIT.

Table 2. Clinicopathological data, immunohistochemistry and c-*kit* gene mutations of testicular GCTs

Case	Age	Histological type	Maximum size (cm)	Ki-67 LI (%)	AI (%)	IHC		Specimens f/p	c-*kit* mutaion	Follow-up
						KIT	SCF			
1	31	mixed GCT	8	37.3	1.9	+[1)]	+[2)]	f, p	wild	AWM, 44 mos
2	60	mixed GCT	8	43.0	2.2	+[1)]	+[2)]	f, p	wild	AW, 3 mos
3	33	seminoma	4.5	50.4	4.3	+	–	f, p	wild	AW, 26 mos
4	22	mixed GCT	7.5	36.2	1.4	+[1)]	+[3)]	f, p	wild	AW, 21 mos
5	31	seminoma	4	43.4	6.1	+	–	f, p	D816V	AW, 20 mos
6	28	seminoma	5	54.6	8.8	+	–	f, p	wild	AW, 16 mos
7	39	mixed GCT	6.5	37.5	2.3	+[1)]	–	f, p	wild	AW, 17 mos
8	46	seminoma	2.5	47.8	2.2	+	–	f, p	wild	AW, 16 mos
9	37	seminoma	7	49.8	6.9	+	–	f, p	wild	AW, 16 mos
10	28	seminoma	12	56.7	5.3	+	–	f, p	wild	AW, 1 mo
11	37	seminoma	11	78.8	5.8	+	–	f, p	wild	AW, 12 mos
12	24	seminoma	5	49.5	4.4	+	–	f, p	wild	AW, 10 mos
13	35	mixed GCT	1.5	53.3	6.2	+[1)]	–	f, p	wild	AW, 9 mos
14	42	seminoma	3.5	37.9	9.0	+	–	f, p	wild	AW, 12 mos
15	21	seminoma	4.5	52.6	5.5	+	–	p	wild	AW, 66 mos
16	51	seminoma	3.5	63.4	5.6	+	–	p	wild	AW, 69 mos
17	38	seminoma	5.5	43.4	5.5	+	–	p	wild	AW, 76 mos
18	24	seminoma	11.5	36.1	4.1	+	–	p	wild	AW, 77 mos
19	38	seminoma	3	39.4	1.9	+	–	p	D816V	AW, 70 mos
20	40	seminoma	7	43.7	2.9	+	–	p	D816H	AW, 60 mos
21	29	seminoma	9	26.2	3.4	+	–	p	wild	AW, 55 mos
22	32	seminoma	3.5	53.4	3.8	+	–	p	wild	AW, 60 mos
23	36	seminoma	7	50.2	3.0	+	–	p	wild	AW, 19 mos
24	34	seminoma	6	43.0	5.0	+	–	p	wild	AW, 18 mos
25	38	seminoma	4.5	43.0	3.2	+	–	p	W557R	AW, 1 mo
26	39	seminoma	7	55.3	10.3	+	–	p	wild	AW, 51 mos
27[4)]	31	seminoma	8	56.4	6.6	+	–	p	wild	DOD, 13 mos
28	31	seminoma	5	53.1	6.6	+	–	p	wild	AW, 44 mos
29	38	seminoma	5	51.0	5.5	+	–	p	wild	AW, 40 mos
30	35	seminoma	4	36.0	4.0	+	–	p	wild	AW, 45 mos
31	34	seminoma	11	52.2	5.2	+	–	p	wild	AW, 34 mos
32	49	seminoma	4	53.8	4.9	+	–	p	wild	AW, 23 mos
33	38	seminoma	2	46.3	6.5	+	–	p	wild	AW, 26 mos
34	24	seminoma	4	53.8	4.6	+	–	p	wild	AW, 1 mo

LI, labeling index; AI, apoptotic index; IHC, immunohistochemistry; f/p, frozen tissues/paraffin sections; +, positive; –, negative; AWM, alive with metastasis; AW, alive and well; DOD, died of disease; mo(s), month(s).
1) Membranous staining was limited to seminoma component.
2) Membranous staining was limited to teratoma component.
3) Cytoplasmic and membranous staining was limited to yolk sac tumor component.
4) This patient died of multiple lung metastases of choriocarcinoma.

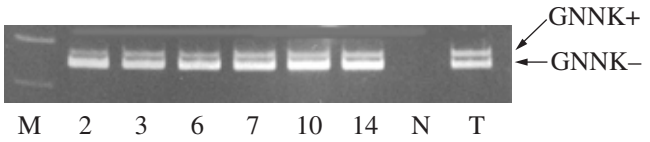

Fig. 2. PCR amplification of the two different isoforms of the c-*kit* gene in GCTs. Coexpression of the GNNK+ and GNNK– isoforms, with dominance of the shorter isoform (GNNK–), is demonstrated (cases 2, 3, 6, 7, 10 and 14). Normal testis (T) was used as control. N, negative control; M, 118- and 72-base pair marker.

29 pure seminomas showed KIT membranous staining. In the 5 mixed GCTs, KIT membranous expression was limited to seminoma components (Fig. 1). In all 14 testicular GCTs and the 1 normal testis examined, coexpression of the GNNK+ and GNNK– isoforms was observed. Dominant expression of GNNK– transcript in GCTs, as compared to that in normal testis, was evident (Fig. 2).

Immunohistochemical expression of SCF was observed in only 3 out of 34 testicular GCTs (cases 1, 2 and 4), and the expression was limited to non-seminoma components of mixed GCTs (2 teratomas and 1 yolk sac tumor) (Fig. 3). The RT-PCR analysis indicated that SCF transcripts were strongly expressed in these three tumors. However, only faint fragments were amplified in the 9 of the 14 GCTs (cases 3, 5–10, 12 and 13) that were not immunoreactive for SCF. Immunohistochemically, nonneoplastic epithelia of rete testis and epididymis were also positive for SCF (Fig. 3).

Mutations of c-*kit* gene in GCTs. Mutations were found in 4 pure seminomas (11.8%) of the 34 testicular GCTs. In case 5, the mutation was detected in both cDNA and genomic DNA. Mutations were detected in exon 11 encoding the juxtamembrane domain and exon 17 encoding the TK2 domain as follows: case 25 showed a point mutation at codon 557 (TGG to CGG) that resulted in a Trp557→Arg (W557R) substitution in exon 11. Cases 5 and 19 showed a point mutation at codon 816 (GAC to GTC) that resulted in an Asp816→Val (D816V) substitution in exon 17 (Fig. 4). Case 20 showed a point mutation at codon 816 (GAC to CAC) that resulted in an Asp816→His (D816H) substitution in exon 17.

Corresponding non-neoplastic tissues from these 4 patients showed only wild-type sequences of the c-*kit* gene, which con-

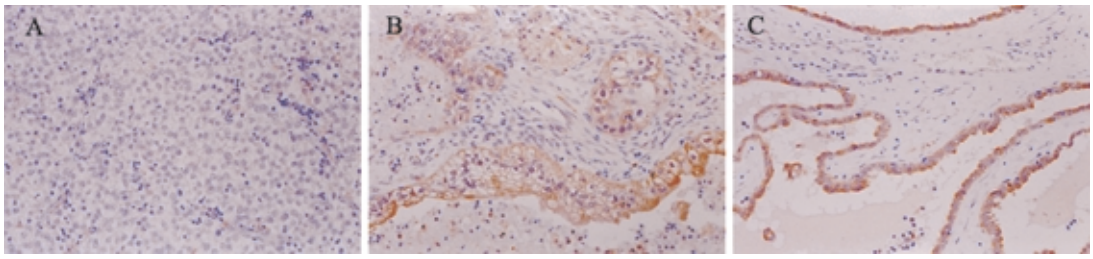

Fig. 3. SCF immunohistochemical staining of case 1 (mixed GCT). (A) Tumor cells in the seminoma component do not express SCF. (B) The teratoma component demonstrates membranous staining of tumor cells. (C) Non-neoplastic epithelia of the rete testis show membranous and intracytoplasmic expression.

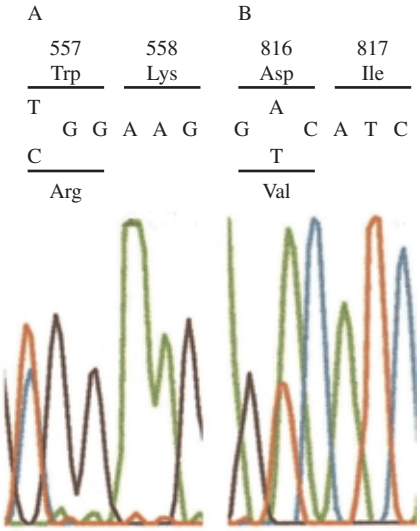

Fig. 4. Genomic sequencing of the c-*kit* gene. (A) Case 25 shows a point mutation at codon 557 in exon 11. Trp557 is changed to Arg. (B) Case 5 demonstrates a point mutation at codon 816 in exon 17. Asp816 is changed to Val.

firmed that the mutations observed in each tumor were somatic.

In case 7, two different sequence alterations were observed. One was observed at codon 541 (ATG to GTG), resulting in a Met541→Val substitution in exon 10 and the other was detected at codon 862 (GAC to GTC), resulting in no amino acid substitution in exon 18. According to the dbSNP Home Page (http://www.ncbi.nlm.nih.gov/SNP/), these alterations are single nucleotide polymorphisms.

GCTs with and without the c-*kit* gene mutation. Clinicopathological data, Ki-67 LI and apoptotic index were compared between patients having testicular GCTs with and without c-*kit* gene mutation (Table 3). No statistically significant difference was found between the two groups.

Discussion

Gain-of-function mutations of the c-*kit* gene have been reported in most GISTs and mast cell tumors. Rubin *et al*. showed c-*kit* gene mutations in 44 (91.7%) of 48 GISTs,[11] and Longley *et al*. found mutations in all patients with adult sporadic mastocytosis.[5] These data indicate that gain-of-function mutations of the c-*kit* gene have a central role in the pathogenesis of these tumors.

In this study, we found several types of c-*kit* gene mutations in 4 pure seminomas (11.8%) out of 34 testicular GCTs that expressed KIT. The frequency is almost equal to that reported by Tian *et al*., who found gain-of-function mutation of exon 17

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

	Mutation (+) (*n*=4)	Mutation (–) (*n*=30)	Statistics
Age (median)	38 (31–40)	34 (21–60)	NS
Maximum size (cm)	4.6±1.7	6.1±2.8	NS
Ki-67 LI (%)	42.4±2.0	48.6±10.1	NS
AI (%)	3.5±1.8	4.9±2.1	NS

NS, not significant; LI, labeling index; AI, apoptotic index.

(D816H) in 8.7% of testicular and ovarian seminomas/dysgerminomas (2 of 23 tumors).[12] The mutations observed in this study were W557R in exon 11, and D816H and D816V in exon 17. The D816H mutation is the same as one found by Tian *et al*.,[12] but the W557R and D816V mutations have not previously been reported in testicular GCTs. Recently, Przygodzki *et al*. reported missense point mutations in exon 17 of the c-*kit* gene (K818R, D820V and N822K) in 3 of 8 primary mediastinal seminomas,[23] but these mutations were not observed in our cases. Even when combination analyses of the whole coding region of c-*kit* cDNAs and the 4 mutational hot spots of the genomic DNAs were done, mutations were found only in exons 11 and 17 in testicular GCTs. These results may imply that initial screenings of mutations in testicular GCTs should be focused on the analysis of exons 11 and 17 of the c-*kit* gene.

In this study, we did not determine the kinase activity and phosphorylation status of KIT, but the D816V mutation has been shown to cause constitutive phosphorylation of KIT *in vitro* and in *de novo* human mast cell neoplasms.[4, 5, 24] D816H KIT has also been shown to be constitutively phosphorylated on tyrosine residues and to have constitutive kinase activity.[12, 24] The W557R mutation has been found in familial and sporadic GISTs.[11, 25] Thus, all 4 mutations identified in the present study are considered to be a gain-of-function type. This result raises the possibility that c-*kit* gene mutation plays an important role in neoplastic behavior in some seminomas, although there are no apparent differences of clinicopathological factors or outcome between patients having seminomas with and without mutations.

The locations of the c-*kit* gene mutations are not random, and they vary according to tumor type. The mutations of the c-*kit* gene in mastocytosis involve predominantly exon 17 encoding the TK2 domain, especially the D816V point mutation,[5] whereas mutations in GISTs involve mainly exon 11 encoding the juxtamembrane domain.[6, 11, 16] Although germline mutation at codon 820 in exon 17 (D820Y) was found in a familial GIST,[10] Kinoshita *et al*. reported that no mutation, including D816V and D820Y, was found in exon 17 in 143 sporadic GISTs.[26] These observations suggest that the effect of each type of c-*kit* gene mutation may vary in different cell types, i.e.,

human mast cells and interstitial cells of Cajal. The c-*kit* gene mutation with oncogenic activity in one cell type may lack such activity in another. Contrary to these observations, it is of interest that c-*kit* gene mutations in both exons 11 and 17 are found in seminomas. Molecular clarification of the downstream signal pathways of KIT, associated with each type of mutation, seems to be needed to understand the differences of mutational hot spots among the tumor types.

In addition to the mutational analysis of the c-*kit* gene, we analyzed the expression of isoforms of the c-*kit* gene and SCF. SCF-KIT autocrine growth regulation has been demonstrated in other tumors, such as small cell lung cancers[13] and Ewing's sarcomas.[14] However, we observed no immunohistochemical expression of SCF in seminomas or seminoma components of mixed GCTs, whereas teratoma and yolk sac tumor components of mixed GCTs are positive for SCF. These results are consistent with those reported by Strohmeyer *et al*. in which SCF mRNA expression was absent in all 8 testicular GCTs including 3 seminomas by northern blot analysis,[22] although Bokemeyer *et al*. observed SCF membranous expression in 7 of 9 seminomas.[21] In this study, epithelia of rete testis and epididymis were also immunohistochemically positive for SCF. In normal testes, Sertoli cells express SCF, and stimulation of KIT by SCF is necessary for maintenance of spermatogenesis.[3] In these results, the faint expression of SCF mRNA in 7 of 9 pure seminomas by RT-PCR may correspond to the expression of SCF by non-neoplastic cells but not by seminomas. Although the SCF-KIT autocrine loop does not seem to play a central role in the pathogenesis of seminomas, it cannot be ruled out that the SCF-KIT juxtacrine loop plays a substantial role in tumorigenesis, in that seminoma cells showed KIT membranous expression and epithelia of rete testis and epididymis, which are near the seminoma, showed SCF staining.

To date, expressions of c-*kit* gene isoforms have not been determined in testicular GCTs. In this study, coexpression of GNNK+/− isoforms was observed by RT-PCR in all testicular GCTs and normal testis, and the preferential expression of GNNK− isoform in GCTs, as compared to that in normal testis, was evident. These two isoforms had been shown to have distinct signal transduction activities in the absence of SCF. The GNNK− but not the GNNK+ isoform showed constitutive tyrosine phosphorylation that was accompanied by a low constitutive level of association with phosphatidylinositol 3-kinase and phospholipase Cγ1.[27] In addition, the GNNK− isoform was also shown to be tumorigenic in NIH3T3 cells, whereas the GNNK+ isoform was not.[28] From these results, preferential expression of GNNK− isoform in testicular GCTs may be seen to serve as an alternative mechanism for increased KIT signaling in testicular GCTs lacking the c-*kit* gene mutation.

STI571 is an ATP-competitive TK inhibitor for c-ABL, BCR/ABL, platelet-derived growth factor receptor and KIT. Recently, significant responses to STI571 have been reported in unresectable and metastatic GISTs that resisted conventional chemotherapy.[17] On the other hand, the application of STI571 to seminomas has never been tried, because even advanced seminomas do well with platinum-based chemotherapy.[29] Evaluation of the c-*kit* gene mutation will be indispensable for such trials, because the presence or absence, and the type of the c-*kit* gene mutations have been reported to produce differences in sensitivity to STI571 *in vitro*; STI571 inhibits wild-type and juxtamembrane domain mutant KIT, whereas TK2 domain mutant KIT is resistant to inhibition by STI571.[18, 19] In contrast, other receptor TK inhibitors, indolinone derivatives, have been reported to inhibit TK2 domain mutant KIT *in vitro*.[30] Thus, it is possible that TK inhibitors, including STI571 and indolinone derivatives, might be effective as an adjuvant therapy for patients with seminomas that are refractory to conventional chemotherapy; such trials could show reduced consequential infertility after chemotherapy or irradiation.

In conclusion, we have found mutations in exons 11 and 17 of the c-*kit* gene in 4 seminomas among 34 testicular GCTs. Although all types of these mutations are considered to be gain-of-function, there were no apparent differences in any clinicopathological factors or outcome between patients with and without mutations. Even the analysis of the whole coding region of the c-*kit* gene revealed mutations in only a minority of seminomas. Moreover, the SCF-KIT autocrine loop has not been observed in this study. KIT activations via preferential expression of GNNK− isoform and/or in a SCF-KIT juxtacrine manner may play more important roles in the development of seminomas. Further studies will be need to clarify the exact role and mechanism of KIT signal pathways in the development of seminomas, and such studies should provide new approaches in therapies for seminomas.

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