ORIGINAL ARTICLE

An Immunohistochemical and Molecular Study of Gastrointestinal Stromal Tumours

Y T Teong, B.Sc.*, S T Teo*, L P Tan, MMed.Sc, B Q WU, M.D***, S C Peh, FRCPath***

*Department of Pathology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia, **Molecular Pathology Unit, Cancer Research Centre, Institute for Medical Research, 50588 Kuala Lumpur, Malaysia, ***Department of Pathology, Peking University, Xue Yuen Lu, Beijing

Summary

Gastrointestinal stromal tumour (GIST) is a rare but most common mesenchymal tumour in the gastrointestinal tract. Although GIST research has been carried out extensively worldwide, it has yet to be studied in Malaysia. To establish the immunohistochemical expression pattern of CD117 (c-KIT), CD34, S-100 and Desmin, the incidence of *c-KIT* and *PDGFRA* genes mutation in GISTs, and correlate it with clinicopathological parameters. Eleven clinically diagnosed GISTs were stained for CD117, CD34, Desmin and S-100 protein by immunohistochemical technique, and *c-KIT* and *PDGFRA* gene mutations were studied by PCR-CSGE-DNA sequencing method. All GISTs (7cases) stain positive for CD117, and co-expressed CD34. None of these cases express Desmin, and only one expressed S-100 protein focally. Fifty-seven percent (4/7 cases) of GIST harboured mutations at exon 11 of *c-KIT* gene, and they were all high risk and malignant cases. No mutation was detected at exons 9, 13 and 17 of *KIT* gene, and exons 12 and 18 of *PDGFRA* gene. Immunohistochemistry using a panel of antibodies shows consistent pattern of CD117 and CD34 expression in GIST, and mutational study may be a useful prognostic marker for kinase inhibitor treatment of GIST.

Key Words: Gastrointestinal stromal tumours, Immunohistochemistry, c-*KIT*, *PDGFRA*

Introduction

Gastrointestinal Stromal Tumor (GIST) is the most common mesenchymal tumour that occurs in the gastrointestinal tract[1]. In the 1940s, GISTs were thought to be smooth muscle tumours. However, with the advent of immunohistochemistry in early 1980s, many of these lesions were found lacking immunophenotypic features of smooth muscle tumour cells. Recently, *c-KIT* mutation[2] and *c-KIT* immunoreactivity in these spindle cell tumours revealed their origin from CD34-positive stem cells that differentiated towards the interstitial cells of Cajal (ICC)[3,4]. ICC expresses c-KIT and the *c-KIT*-stem cell factor interaction is pivotal in ICC maturation[5]. Since KIT expression is frequent in GISTs, it is now used as a marker for GIST[6].

C-KIT is a type III transmembrane tyrosine kinase receptor. The *c-KIT* receptor and its cognate ligand play a critical role in hematopoiesis, melanogenesis and gametogenesis[7] by involving in different signaling pathways such as the Ras/Rrk pathway, the JAK/STAT pathway and the phosphotidylinositol 3-kinase (PI-3 kinase)/Akt pathway, through its kinase domains[8,9]. The activity and expression of KIT is tightly regulated in normal cells, but it is deregulated in cancer cells. Oncogenic activation and overexpression of KIT protein is closely related to mutations in KIT, the proto-oncogene[10,11].

C-KIT is the cellular homologue of the v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene, and is located in the long arm of chromosome 4. There are

This article was accepted: 1 November 2006

Corresponding Author: Suat-Cheng Peh, Department of Pathology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

several types of *c-KIT* mutations being identified in the past, with mutation at exon 11 (associated with the juxtamembrane domain of receptor) being the most common type in GIST. Mutations at exon 9 (the extracellular domain), exon 13 (the kinase I domain) and exon 17 (activation loop) were also identified[12-15]. The oncogenic activation of *c-KIT* protein in GIST is due to structural changes in the *c-KIT* proteins that make it favours receptor oligomerization and cross-phosphorylation (activation) even in the absence of its ligand[16].

Recently, mutations of an alternative oncogene, the platelet-derived growth factor receptor alpha *(PDGFRA)*, was also observed in GIST[17,18]. Like *c-KIT*, *PDGFRA* is also a type III transmembrane tyrosine kinase receptor. Mutations in exon 12 (the juxtamembrane domain) and 18 (tyrosine kinase domain) of *PDGFRA* are more common[19]. Mutations of *KIT* and *PDGFRA* had been identified to be mutually exclusive[20-22]. There was a study showing that GISTs with *PDGFRA* mutations have a better prognosis than those with *c-KIT* mutations[23].

Investigation on kinase expression and kinase gene mutation is important as *c-KIT* and *PDGFRA* are speculated to be a therapeutic target of imatinib, which is a biological inhibitor of kinases. GIST patients with *c-KIT* mutation at exon 9 and exon 11 are equally sensitive to imatinib, while those with mutations of other exons acquire resistance to imatinib. Different responses to imatinib treatment are also seen in patients with different mutant isoforms of *PDGFRA*[24].

Conventional histopathological assessments such as tumour size, mitotic index, histological subtype and immunohistochemical staining with a panel of antibodies have been used to confirm GIST and determine its malignant status[6,19,25]. In the recent years, advancement in genetics has enabled the mutational study on GISTs, and as a consequence, higher accuracy of diagnosis and prognosis of GISTs can be achieved[26,27]. The aim of this study is to establish and correlate the genetic alteration patterns of *c-KIT* and *PDGFRA*, and the expression pattern of CD117 (c-KIT), CD34, S-100 and Desmin in GIST.

Materials and Methods

Samples

Formalin-fixed, paraffin-embedded tissue blocks of clinically diagnosed GIST cases from year 2000 to 2004 were collected from the archive. Serial sections from the blocks of these cases were stained with hematoxylin-eosin and a panel of antibodies with immunohistochemical method. Pathological risk factor was recorded as very low risk, low risk, intermediate risk and high risk according to Fletcher classification[28], and GIST was histologically classified as epithelioid, spindle and mixed type (contain more than 20% of another type).

Immunohistochemical Analysis

Three μm thick sections were quenched in 3% hydrogen peroxide in methanol to block endogenous peroxidase activity and subjected to microwave treatment to retrieve antigen for the staining procedure for CD117 (c-KIT), CD34 and Desmin. No antigen retrieval is needed for S-100. After conditioning in Tris-buffered solution (TBS, 8 g/L NaCl, 0.6 g/L Tris-base, 3.8 N HCl, pH 7.6), the sections were incubated with respective primary antibody: anti-c-KIT (CD117) (1:50; DakoCytomation, USA), anti-CD34 (1:50, QBEnd-10; DakoCytomation, USA), anti-Desmin (1:50, D33; Dako A/S, Denmark) and S-100 (1:1000, Dako A/S, Denmark). After incubation, the sections were first washed in TBS, and then immersed in ChemMate DAKO Envision (HRP, Rabbit/Mouse) solution, followed by visualization with Diaminobenzidine of ChemMate DAKO EnVision detection kit (DakoCytomation, Denmark) according to the manufacturer's instruction. The sections were slightly counterstained in Mayer's hematoxylin, rinsed with water, dehydrated in a series of alcohol and xylene and mounted. Positive expression of CD117 was used as the confirmative criterion for GIST. Intestinal cells of Cajal, endothelial cells of blood vessel and smooth muscle of intestine served as internal controls for CD117, CD34 and Desmin respectively.

DNA Extraction

The genomic DNA was extracted from formalin-fixed paraffin-embedded tissue blocks. Briefly, pieces of 5μm-thick sections were cut using sterilised microtome (Leica RM2135, Germany) and placed into 1.5 mL-microcentrifuge tubes. The sections were deparaffinised two times with xylene, and washed twice with absolute ethanol. The tissue was then dried in the air for 30 minutes before subjecting to digestion. Proteinase K digestion in 1X PCR buffer (200ug/mL) was carried out at 55°C overnight, and then inactivated at 95°C for 10 minutes. Any remaining tissue debris was eliminated by centrifugation at 12000 rpm for 10 minutes, and the supernatant was used directly for polymerase chain reaction (PCR).

Polymerase Chain Reaction

Exon 9, 11, 13 and 17 of *c-KIT* gene and exon 12 and 18 of *PDGFRA* gene were amplified by PCR, using the oligonucleotide primer pairs listed in Table I. The 25uL PCR reaction mixture contains 1X PCR buffer, 5 mM $MgCl_2$, 0.2 mM dNTPs mix, 1U HotStarTaq polymerase (Qiagen GmbH, Hildon, Germany), 1.5 μM each of forward and reverse primers and 1% DNA template. Cycling conditions for the PCR were as follow: 95°C for 15 minutes; followed by 40 cycles of: 95°C for 30 seconds, annealing temperature (52°C - 57°C, depending on the primers shown in Table I) for 30 seconds and 72°C for 30 seconds; and final extension at 72°C for 4 minutes. The PCR products were then kept at 4°C.

Conformation Sensitive Gel Electrophoresis

Mutational screening by Conformation Sensitive Gel Electrophoresis (CSGE) was performed according to Ganguly *et al.*[29] and Korkko *et al.*[30] with minor modifications. Briefly, 20 μl PCR product was mixed with EDTA to a final concentration of 10 mM, heated at 95°C for five minutes and incubated at 65°C for one hour to generate heteroduplex (if there is mutation). Samples were then mixed with same volume of loading dye (40% w/v sucrose, 0.25% xylene-glycol and 0.25% bromophenol blue) and electrophoresed on a 10% polyacylamide (29:1 acrylamide to bisacrylamide) with 1X TTE running buffer (8.8 mM Tris, 2.9 mM Taurine, 0.02 mM EDTA, pH 9.0) at constant 800 V, 45W for four hours, by using model S2 Sequencing gel electrophoresis apparatus (Life Technologies Inc., Gaithersburg, USA.). The gel was stained with SYBR Gold (Molecular Probe, Invitrogen, USA) and visualized in a gel documentation system (UVP).

DNA Sequencing

Samples with heteroduplexes observed in CSGE were subjected to automated sequencing. The PCR product was first purified with QIAquick PCR purification kit (Qiagen Inc, Valencia, CA) and sequencing of purified PCR products was performed using ABI PRISM BigDye Terminator Cycle Sequencing (Perkin Elmer, Foster City, CA, USA) in ABI 310 Genetic Analyzer (Applied Biosystem, Perkin Elmer, Foster City, CA, USA.). All mutations were confirmed with at least two times of independent sequencing reactions.

Results

Sample Data

From a total of 11 clinically-diagnosed GISTs, three cases were incisional biopsies and the remaining were excised tumors. The ages of patients at the time of diagnosis ranged from 25 to 76 years (mean = 57 years). There were six males and six females; five Chinese, three Malay and three Indian.

Immunohistochemical Expression Profile

Out of these 11 cases, 7 were confirmed as GIST, 3 were non-GIST, and 1 remained indeterminate (tissue was non-reactive to immunohistochemical stains). All GIST cases co-expressed CD117 and CD34 (Figures 1d, 1e), and there were 5 spindle, 1 epithelioid, and 1 mixed histology types (Table II and Figures 1a, 1b, 1c). The CD117 staining was strong, and was localized in cytoplasm and membrane (Figure 1d). Only one case (Case 7) expressed S-100 protein focally, and none of them expressed Desmin. All the non-GIST cases did not express CD117 and CD34, but had either strong Desmin or S-100 expression. They were re-diagnosed as inflammatory pseudo-tumor (Case 9), smooth muscle tumor (Case 10) and peripheral nerve sheath tumor (Case 11). All non-GIST cases were excluded from further study except case 9, which was used as negative control.

Clinicopathologic Features of GISTs

Among the confirmed GIST patients, four were females and three were males. Three of them were Chinese, two were Malay, and two were Indian. The mean age of patients at the age of diagnosis was 60 years (range: 29-76 years). The majority of them aged above 50 years (6/7), presented in the stomach (5/7), and spindle morphology was more common (5/7). Four out of the seven cases were high risk-GIST based on Fletcher classification.

Evaluation of Mutations in *c-KIT* and *PDGFRA* Genes

Mutation screening of *c-KIT* and *PDGRFA* genes were carried out on all confirmed GIST cases, the indeterminate (Case 8) and a non-GIST (Case 9) using PCR-CSGE-DNA sequencing method. A total of 54 PCR products were screened for the presence of heteroduplex in CSGE. Four heteroduplices were observed for exon 11 of *KIT* and no heteroduplex was observed for other exons. DNA sequencing revealed four different types of mutation for the four heteroduplices (Table III). Case 1 has a 9 base-pair deletion (75685_75693GAAGGTTGT) resulting in deletion of 3 amino acid residues, W557 to V560, and an insertion of Cysteine residue (W557-V560delinsC). Case 3 has a 3 base-pair in-frame deletion (75749_75751delGAT), resulting in a deletion of aspartic acid residue (D579 del). Case 5 has a 6 base-

pair deletion (75688_75693delGGTTGT), resulting in deletion of 3 amino acid residues, K558 to V560, and an insertion of Asparagine residue (K558_V560delinsN). Case 6 has a 52 base-pair deletion and a single T insertion (75679_75730delinsT), which results in a deletion of 17 amino acid residues, Q556 to D572 (Q556_D572del). Deletion is the common mutation among the four cases, and all of them had in-frame deletion in amino acid level.

Discussion

Molecular and immunohistochemical studies on GISTs had been extensively carried out in United States, Japan, China and many European countries. However, the study of this disease has not been carried out in Malaysia. Early studies aimed to characterize GIST and to distinguish it from smooth muscle tumours. Later, it proceeded to the assessment of prognostic criteria. As imatinib mesylate and other kinase inhibitors were proposed to be used as adjuvant therapy for GISTs that harbor *c-KIT* gene mutation, the studies of this tumour has shifted from prediction of malignancy to proper selection of patient for adjuvant chemotherapy.

There were only 11 cases of GISTs reported in University of Malaya Medical Centre from year 2000 to 2004, which indicates that GIST is an uncommon disease. After re-assessment by pathologist using standard criteria[31], only seven of them are confirmed GISTs, and one of them remain indeterminate. All GISTs show strong expression of *c-KIT* protein regardless of tumour site, histological subtype, and benign or malignant, which is in agreement with previous study[32]. Most of the GISTs (86%, 6/7) examined co-expressed CD34, consistent with previous reports[14,15,19]. None of the cases expressed Desmin, and only one of them expressed S-100 protein focally, indicating that Desmin and S-100 expression are uncommon in GISTs, in accordance with other reports[14, 19, 28].

Similar to previous studies[33,34], our small series of cases showed 57% (4/7) of GIST cases harbored *c-KIT* gene mutation at exon 11. The reported mutation frequencies for *c-KIT* gene in GISTs range from 15-80%[14,15,27,35,36] and the wide differences are believed to be mainly due to the proportion of malignant and high-risk cases in the studies. Our study showed that all GISTs that harbour mutations in exon 11 of *c-KIT* gene are high risk GISTs and malignant cases by histomorphological criteria, while none of the non-GIST or low risk-GISTs have mutation.

Previous studies show that codon 550 to 560 of *c-KIT* gene is the mutation hot spot for GISTs[15,32,35]. Two out of 4 mutations (Case 1 and 5) detected in our study were located at this hot spot. There is one mutation (Case 6), which starts at the hot spot and extends to codon 573. This mutation involved a large deletion (52bp) and a single T insertion, leading to the loss of 17 amino acids residues. We also found a GAT deletion

Table I: Oligonucleotide Primers for *c-KIT* and *PDGFRA* genes

	Primer (5' -> 3')		Annealing temperature (°C)	Fragment Size (bp)
	Forward	Reverse		
c-KIT				
9	TCCTAGAGTAAGCCAGGGCTT	TGGTAGACAGAGCCTAAACATCC	55	284
11	CTGAGACAATAATTATTAAAAGGTGA	TTATGTGTACCCAAAAAGGTGACATGG	52	227
13	ACTGCATGCGCTTGACATCAGTTTGCCAG	AAAAGGCAGCTTGGACACGGCTTTA	52	203
17	ACAAGTTAAAATGAATTTAAATGGT	TTGAAACTAAAAATCCTTTGC	52	224
PDGFRA				
12	TCCAGTCACTGTGCTGCTTC	GCAAGGGAAAAGGGAGTCTT	65	260
18	ACCATGGATCAGCCAGTCTTG	TGGGAGGATGAGCCTGTCC	56	250

Abbreviation: PDGFRA, platelet-derived growth factor receptor alpha.

Table II: Clinicopathological data of GISTS

No	Race	Age	Sex	Site	Mitotic count	Tumour size (cm)	Risk Factor	Malignancy	CD117	CD34	Desmin	S-100	Histology	Diagnosis	Mutation
1	C	52	F	S	13/50	14 x 12 x 10	H	M	2+	3+	-	-	Mixed	GIST	9 bp del
2	M	61	M	S	4/50	4 x 3	L	B	+	+	-	-	Spindle	GIST	-
3	C	67	F	S	9/50	10 x 6 x 2	H	M	3+	2-3+	-	-	Spindle	GIST	3 bp del
4	C	76	F	S	<5/50	8cm (diameter)	I	B	2+	2-3+	-	-	Epithelioid	GIST	-
5A	I	66	M	R	44/50	3 x 3 x 3*	H	M	2+	3+	-	-	Spindle	GIST	6 bp del
5B				R	16/50	6 x 5 x4	H		3+	3+	-	-	Spindle	GIST	
6	M	29	F	SI	6/50	5 x 3 x 3	H	M	3+	-/+	-	-	Spindle	GIST	52 bp del + 1 bp ins
7	I	66	M	S	est. <2/50	0.5*	VL	B	3+	3+	-	+	Spindle	GIST	-
8	I	48	M	D	†	0.2*	N/A	B	NR	-	-	-	Epithelioid	i.d.	-
9	C	25	F	S	N/A	8 x 6.5 x 7	N/A	N/A	-	-	-	-/+	N/A	IP	N/A
10	M	62	M	SI	N/A	6 x 5.5 x 3.5	N/A	N/A	N/A	-	3+	-	N/A	SMT	N/A
11	C	71	M	S	N/A	1 x 0.5 x 0.5	N/A	N/A	-	-	-/+	3+	N/A	PNST	N/A

Abbreviation: S, stomach; R, rectum; D, duodenum; SI, small intestine; †, high mitosis but little biopsy; * in aggregates; H, high; I, intermediate; L, low; VL, very low; N/A, not available; NR, not reactive; M, malignant; PM, potential malignant; B, benign; i.d., Indeterminate; IP, Inflammatory Pseudotumor; SMT, smooth muscle tumour; PNST, peripheral nerve sheath tumour.

Table III: Amino acid sequence encoded by wild type and mutant *c-KIT* exon 11.

	550	560	570	580
Wildtype	KPMYEVQWKV	VEEINGNNYV	YIDPTQLPYD	HKWEFPRNRL
Case 1	KPMVEVQC - -	- EEINGNNYV	YIDPTQLPYD	HKWEFPRNRL
Case 3	KPMYEVQWKV	VEEINGNNYV	YIDPTQLPY -	HKWEFPRNRL
Case 5	KPMYEVQWN -	- EEINGNNYV	YIDPTQLPYD	HKWEFPRNRL
Case 6	KPMYEV- - - -	- - - - - - - - - -	- - - PTQLPYD	HKWEFPRNRL

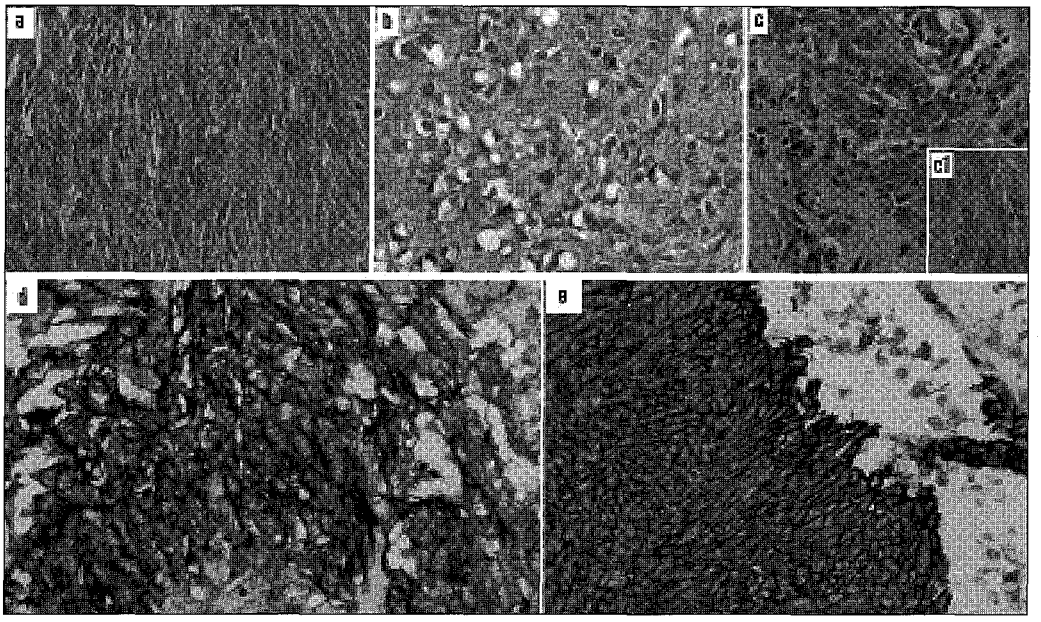

Fig 1: Histological subtype of GIST. (a) spindle type, (b) epithelioid type, (c) mixed spindle-epithelioid type; immunohistochemical staining profile of GIST showing (d) CD117 expression, and (e) CD34 expression.

(codon 579) that located beyond the hot spot area of c-*KIT* gene, which led to the loss of an aspartic acid residue (case 3) for the protein. As described in previous study[11], KIT^{del579} was a 'gain-of-function mutation that eventually resulted in constitutive activation of c-*KIT* protein, hence tumorigenesis of GIST.

No mutation was detected in other exons of *c-KIT* gene. This suggested that mutation in these exons was less common than that in exon 11. Due to the small sample size, correlation of mutation spectrum with clinical data cannot be determined.

c-KIT and *PDGFRA* gene mutations were claimed to be an important predictive value of clinical response of imatinib mesylate on GISTs. GIST patients with mutations in exon 11 of *c-KIT* were reported to have better response and better survival under imatinib mesylate treatment when compared to those with mutations in exon 9 of *c-KIT*, or with no detectable mutations[24,37]. Our study showed exon 11 *c-KIT* mutation rate of 57% among the GIST cases examined, and hence it may be more beneficial to our local GIST patients if imatinib mesylate is instituted for the treatment of GIST with exon 11 *c-KIT* mutation.

In conclusion, immunohistochemical staining using a panel of antibodies are helpful for making the diagnosis of GIST. Not all *c-KIT*-positive GISTs harboured *c-KIT* mutation, and all of these cases with mutations (57%) were of poor prognostic morphology when compared to those without mutation.

References

1. Miettinen M, Monihan JM, Sarlomo-Rikala M *et al.* Gastrointestinal stromal tumors/smooth muscle tumors (GISTs) primary in the omentum and mesentery: clinicopathologic and immunohistochemical study of 26 cases. Am J Surg Pathol 1999; 23: 1109-18.
2. Hirota S, Isozaki K, Moriyama Y *et al.* Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 1998; 279: 577-80.
3. Wang L, Vargas H, and French SW. Cellular origin of gastrointestinal stromal Tumors—A study of 27 cases. Arch Pathol Lab Med 2000; 124: 1471-5.
4. Kindblom L-G, Remotti HE, Aldenborg F, and Meis-Kindblom JM. Gastrointestinal Pacemaker Cell Tumor (GIPACT)—Gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am J Pathol 1998; 152: 1259-69.
5. Vliagoftis H, Worobec AS, and Metcalfe DD. The proto-oncogene c-kit and c-kit ligand in human disease. J Allergy Clin Immunol 1997; 100: 435-40.
6. Sarlomo-Rikala M, Kovatich AJ, Barusevicius A, and Miettinen M. CD117: a sensitive marker for gastrointestinal stromal tumors that is more specific than CD34. Mod Pathol 1998; 11: 728-34.
7. Lammie A, Drobnjak M, Gerald W, Saad A, Cote R, and Cordon-Cardo C. Expression of c-kit and kit ligand proteins in normal human tissues. J Histochem Cytochem 1994; 42: 1417-25.
8. Lennartsson J, Voytyuk O, Heiss E, Sundberg C, Sun J, and Rönnstrand L. C-kit signal transduction and involvement in cancer. Cancer Therapy 2005; 3: 5-28.
9. Hong L, Munugalavadla V, and Kapur R. C-Kit mediated overlapping and unique functional and biochemical outcomes via diverse signaling pathways. Mol Cell Biol 2004; 24: 1401-10.
10. Kang HJ, Nam SW, Kim H *et al.* Correlation of KIT and platelet-derived growth factor receptor a mutations with gene activation and expression profiles in gastrointestinal stromal tumors. Oncogene 2005; 24: 1066-74.
11. Nakahara M, Isozaki K, Hirota S *et al.* A novel gain-of-function mutation of c-kit gene in gastrointestinal stromal tumors. Gastroenterology 1998; 115: 1090-5.
12. Lux ML, Rubin BP, Biase TL *et al.* KIT extracellular and kinase domain mutations in gastrointestinal stromal tumors. Am J Pathol 2000; 156: 791-5.
13. Hirota S, Nishida T, Isozaki K *et al.* Gain-of-function mutation at the of extracellular domain of KIT in gastrointestinal stromal tumours. J Pathol 2001; 193: 505-10.
14. Koay MHE, Goh YW, Iacopetta B *et al.* Gastrointestinal stromal tumours (GIST): a clinicopathological and molecular study of 66 cases. Pathology 2005; 37: 22-31.
15. Rubin BP, Singer S, Tsao C *et al.* KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. Cancer Res 2001; 61: 8118-21.
16. Heinrich MC, Rubin BP, Longley BJ, and Fletcher JA. Biology and genetic aspects of gastrointestinal stromal tumors: KIT activation and cytogenetic alterations. Hum Pathol 2002; 33: 484-95.
17. Hirota S, Ohashi A, Nishida T *et al.* Gain-of-function mutations of platelet-derived growth factor receptor a gene in gastrointestinal stromal tumors. Gastroenterology 2003; 125: 660-7.
18. Pauls K, Merkelbach-Bruse S, Thal D, Büttner R and Wardelmann E. PDGFRa- and c-kit-mutated gastrointestinal stromal tumours (GISTs) are characterized by distinctive histological and immunohistochemical features. Histopathology 2005; 46: 166-75.
19. Miettinen M, Sobin LH and Lasota J. Gastrointestinal stromal tumors of the stomach – A clinicopathologic, immunohistochemical, and molecular genetic study of 1765 cases with long-term follow-up. Am J Surg Pathol 2005; 29: 52-68.
20. Heinrich MC, Corless CL, Duensing A *et al.* PDGFRA activating mutations in gastrointestinal stromal tumors. Science 2003; 299: 708-10.
21. Wasag B, Debiec-Rychter M, Pauwels P *et al.* Differential expression of KIT/PDGFRA mutant isoforms in epitheliod and mixed variants of gastrointestinal stromal tumors depends predominantly on the tumor site. Mod Pathol 2004; 17: 889-94.
22. Medeiros F, Corless CL, Duensing A *et al.* KIT-negative gastrointestinal stromal tumors: a proof of concept and therapeutic implications. Am J Surg Pathol 2004; 28: 889-94.
23. Lasota J, Dansonka-Mieszkowska A, Sobin LH, and Miettinen M. A great majority of GISTs with PDGFRA mutations represent gastric tumors of low or not malignant potential. Lab Invest 2004; 84 : 874-83.

24. Heinrich MC, Corless CL, Demetri GD *et al.* Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol 2003; 21: 4342-9.

25. Yokoi K, Tanaka N, Shoji K *et al.* A study of histopathological assessment criteria for assessing malignancy of gastrointestinal stromal tumor, from a clinical standpoint. J Gastroenterol 2005; 40: 467-73.

26. Singer S, Rubin BP, Lux ML *et al.* Prognostic value of KIT mutation type, mitotic activity, and histologic subtype in gastrointestinal stromal tumors. J Clin Oncology 2002; 20: 3898-905.

27. Kim TW, Lee H, Kang YK *et al.* Prognostic significance of c-kit mutation in localized gastrointestinal stromal tumors. Clin Cancer Res 2004; 10: 3076-81.

28. Fletcher CDM, Berman JJ, Corless C *et al.* Diagnosis of gastrointestinal stromal tumors: A consensus approach. Hum Pathol 2002; 33: 459-65.

29. Ganguly A, Rock MJ, and Prockop J. Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: Evidence for solvent-induced bends in DNA heteroduplexes. Proc Natl Acad Sci USA 1993; 90: 10325-329.

30. Körkkö J, Annunen S, Pihlajamaa T, Prockop DJ and Ala-Kokko L. Conformation sensitive gel electrophoresis for single and accurate detection of mutations: comparison with denaturing gradient gel electrophoresis and nucleotide sequencing. Proc Natl Acad Sci USA 1998; 95: 1681-5.

31. Blay J-Y, Bonvalot S, Casali P *et al.* Consensus meeting for the management of gastrointestinal stromal tumors: report of the GIST Consensus Conference of 20-21 March 2004, under the auspices of ESMO. Ann Oncol 2005; 16: 566-78.

32. Feng F, Liu XH, Xie Q, Liu WQ, Bai CG, and Ma DL. Expression and mutation of c-kit gene in gastrointestinal stromal tumors. World J Gastroenterology 2003; 9: 2548-51.

33. Lasota J, Jasinski M, Sarlomo-Rikala M, and Miettinen M. Mutations in exon 11 of c-kit occur preferentially in malignant versus benign gastrointestinal stromal tumor and do not occur in leiomyomas and leiomyosarcomas. Am J Pathol 1999; 154: 53-60.

34. Taniguchi M, Nishida T, Hirota S *et al.* Effect of c-kit mutations on prognosis of gastrointestinal stromal tumors. Cancer Res 1999; 59: 4297-300.

35. Hou YY, Tan YS, Sun MH *et al.* C-kit gene mutation in human gastrointestinal stromal tumors. World J Gastroenterol 2004; 10: 1310-4.

36. Sakurai S, Fukasawa T, Chong J-M, Tanaka A, and Fukayama M. C-kit gene abnormalities in gastrointertinal stromal tumors (tumors of interstitial cells of Cajal). Jpn J Cancer Res 1999; 90: 1321-8.

37. Debiec-Rychter M, Dumez H, Judson I *et al.* Use of c-kit/PDGFRA mutational analysis to predict the clinical response to imatinib in patients with advanced gastrointestinal stromal tumours entered on phase I and phase II studies of the EORTC Soft Tissue and Bone Sarcoma Group. Eur J Cancer 2004; 40: 689-95.