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KIT and PDGFRA mutation analysis of GIST

ZAFER KÜÇÜKODACI[1], GAYE G. TEZEL[2], AKTUĞ H. ŞİMŞEK[1], GÖKHAN GEDİKOĞLU[2], HÜSEYİN BALOĞLU[1]

[1]GATA Haydarpaşa Training Hospital, Department of Pathology, Istanbul, [2]Hacettepe University Medical School, Department of Pathology, Ankara-Turkey

ABSTRACT

In this study, KIT and PDGFRA gene mutations which play a crucial role in biologic behavior and treatment of gastrointestinal stromal tumors that is the most commonly seen mesenchymal tumors in the gastrointestinal system were evaluated in a series of 29 cases. Total genomic DNA extracted from the paraffin embedded tumor tissues of 29 cases were used as template DNA. With designed primers for exons of KIT and PDGFRA genes, target DNA was amplified using optimized PCR techniques. Amplicons were screened for mutation. After screening, the exons suspected of having undergone mutation were subjected to DNA sequencing analysis. In 17 cases, quality and amount of extracted DNA obtained from archival blocks were sufficient. In only 11 cases, all 6 targeted exons could have been amplified efficiently for further applications and mutations were observed in 4 of these 11 patients. In conclusion, we suggest that it would be reasonable to provide screening tests for mutation in exons 9, 11, 13, 17 for KIT gene and exons 12, 14, 18 for PDGFRA gene following the establishment of the diagnosis of GIST. [Turk J Cancer 2009;39(1):11-17]

KEY WORDS: KIT, PDGFRA, GIST

INTRODUCTION

Gastrointestinal stromal tumors (GIST) are the most common primary mesenchymal tumors of the gastrointestinal tract. They are mostly originated from the stomach (%40-70). Less commonly they may be seen in the small intestines (%20-50), colon-rectum (%5-15) and esophagus (<%2). They are very rare outside the gastrointestinal tract (1,2). The tumor is considered to be derived from the interstitial cells of Cajal. Whereas the tumor cells express CD117 and CD34 antigens, like Cajal cells, they do not express desmin and S100 (1).

It is hard to predict the biologic behaviour of the GIST. Its prognosis may vary according to tumor localization, size, morphologic type, mitotic activity and the presence of metastasis (3).

c-KIT and PDGFRA are the members of tyrosine kinase receptor family (1). KIT and PDGFRA gene mutations are the major genetic alterations seen in GIST (1). In 90% of the tumors, one of these genetic mutations is present (4,5). KIT and PDGFRA gene mutations are not only associated with specific cell morphology, histologic phenotype, metastasis and prognosis, but they are also highly crucial in determining the therapeutic targets. The detection of KIT and PDGFRA gene mutations in GISTs enabled the application of imatinib, sunitinib and other tyrosine kinase inhibitors in the treatment of the tumors.

MATERIALS AND METHODS

DNA extraction

In this study, total genomic DNA extracted from paraffin embedded tumor tissues of 29 cases diagnosed as GIST, 20 cases from Hacettepe University Medical School Department of Pathology and 9 cases from GATA Haydarpaşa Training Hospital Department of Pathology, was studied. Clinical prognostic parameters of these cases, such as age, gender, localization, and morphologic prognostic parameters, like tumor size, cellularity, pleomorphism, cell type, mitotic count and the presence of necrosis are shown in table 1. Most former paraffin block was embedded 25 years ago. For DNA extraction, conventional phenol/cloroform/isoamyl alcohol method (n=20) and filtered spin-column method (n=9) (QIAamp DNA mini Kit, QIAGEN, Germany) was applied (6). In 12 cases, sufficient quantity and quality of DNA extraction was unsuccessful. In 17 cases having sufficient amount of DNA (>1µg) and appropriate DNA quality (mostly >500 bp), target exons were amplified.

The amplification of KIT and PDGFRA target exons

Using designed primers for KIT gene exons 11, 9, 13, 17 and PDGFRA gene exons 18, 12, 14, all of the target exons from extracted DNA of the cases were amplified with the polymerase chain reaction (PCR). Designed primers for target gene exons and details of the PCR method is shown below (Table 2 and Table 3). In 6 cases out of 17, none of the exons were amplified in PCR reaction.

Mutation analysis of the amplicons

In 11 cases out of 17, KIT gene exons 11, 9, 13, 17 and PDGFRA gene exons 18, 12, 14 were amplified in PCR reaction. For mutation screening in these amplicons;

a) The heteroduplexes formed by amplicons and their wild type homologous were electrophoresed and their agarose gel patterns were examined after surveyor DNA restriction enzyme reaction (SURVEYOR Mutation Detection Kit for Standard Gel Electrophoresis. Transgenomics, USA).

b) Conformation diversity of single strand DNA of the cases and their wild type homologous were comparatively evaluated using their polyacrylamide gel patterns (7,8).

Of 11 cases searched for mutation, in 4 cases, mutation findings were detected (Figure 1). These mutations were confirmed by sequence analysis and mutation types were identified (Figure 1).

RESULTS

In only 17 cases out of 29, appropriate quality and sufficient amount of DNA was extracted for PCR reactions. Using the extracted DNA from 17 cases as template, target exons were amplified with PCR reaction in 11 cases, of which mutation findings shown in figure 1 were detected in only 4 cases.

DISCUSSION

From our series of 29 cases, sufficient amount and appropriate quality of DNA was obtained in 17 cases. Twelve cases from which DNA can not be extracted were embedded in paraffin former than 10 years, and also phenol/chloroform/isoamyl alcohol method had been applied for DNA extraction. The reason for insufficient amount and inappropriate quality of DNA extraction may be requirement of more paraffin embedded tissue for phenol/chloroform/isoamyl alcohol method, as well as formalin fixation and older paraffin block archive years.

In spite of various modifications, in 6 cases out of 17, amplification was not successful in PCR reaction. Also, in all these six cases, phenol/chloroform/isoamyl alcohol method was used for DNA extraction. The reason for unsuccessful DNA extraction in this method may be insufficient elimination of PCR inhibitors, such as DNA polymerase enzyme inhibitors.

Mutations were detected in 4 of 11cases, in which all target exons were successfully amplified. In three cases out of 4, mutation was in KIT gene, whereas it was in PDGFRA gene in one case. In literature, the mutation rates vary between 30-95% for KIT gene, and 5-20% for PDGFRA gene (1,4, 9-11). KIT and PDGFRA encodes homologous of tyrosine kinase receptor proteins. Both of them are pericentromerically located on 4q12 (4). Tyrosine kinase receptor controls cell proliferation, differentiation and adaptation. Mutations of KIT and PDGFRA genes in GISTs may cause uncontrolled cell prolifera-

tion and inhibition of apoptosis by producing continuous signals without ligand of the receptor. In decreasing frequency, these mutations are seen in exons 11, 9, 13 and 17 for KIT gene, and in exons 18, 12 and 14 for PDGFRA gene (3,12,13). In our study, all 3 cases having KIT gene mutation were seen in exon 11, and only one case with PDGFRA gene mutation was in exon 18. Mutations of KIT and PDGFRA genes are closely related to each other in terms of histological phenotype, prognosis as well as treatment choice (5). KIT exon 11 mutation is the most common one seen in GISTs and it appears as deletion, point mutation and duplication of 3' end. Exon 9 mutations are usually duplication of 6 nucleotide sequences. Exon 13 and 17 mutations are almost always point mutations and their frequency is less than 1-2% (3,5). In this study, two of the 3 KIT mutations are deletion and the re-

Table 1
Cinical and morphological features of 29 GIST cases that were analysed for mutation

No	Age	G	Localization	Size	Necrosis	C	P	Cell type	Mitosis/50 HPF
1**	50	M	Mesentery	3	Present	++	++	S	16
2**	40	M	Stomach	8	Absent	+	++	S	12
3*	41	M	Jejunum-Ileum	10	Present	+	++	S	16
4*	65	M	Colon	7	Absent	+	+	S	27
5*	57	F	Colon	12	Present	++	+	S	5
6**	86	F	Ileum	6	Present	+	+	S	1
7*	40	M	Jejunum-Ileum	7	Absent	++	+	S	1
8*	28	F	Mesentery	7	Present	+	++	S	11
9*	65	M	Colon	11	Present	+	+++	S	10
10*	66	M	Stomach	11	Present	++	+	S	20
11*	42	F	Jejunum-Ileum	2	Present	+	+	S	50
12*	75	M	Mesentery	16	Present	+	+	S	2
13*	33	F	Duodenum	7	Present	+	+++	S+Ep	1
14**	50	F	Stomach	20	Present	++	+	S	2
15	61	M	Stomach	5	Absent	+	+	S	2
16**	69	M	Stomach	3,5	Absent	++	+	S	18
17*	62	F	Colon	10	Absent	+	++	S	4
18**	72	F	Stomach	6	Absent	+	+	S	2
19	54	F	Colon	7	Absent	+	+	S	1
20*	73	F	Ileum	10	Present	++	+	S	2
21†	67	F	Colon	9	Present	+++	+++	S	8
22	78	F	Ileum	5	Absent	+	+	S	3
23‡	59	M	Stomach	7	Present	++	++	S+Ep	12
24†	55	M	Colon	14	Present	++	+	S	7
25†	69	F	Ileum	11	Present	+	+	S	3
26	70	M	Mesentery	9	Absent	+	+	S	3
27	65	M	Stomach	5	Absent	+	+	S	3
28	48	M	Colon	10	Present	+	++	S	8
29	60	M	Colon	17	Present	++	++	S	14

G: Gender; M: Male; F: Female; C: Cellularity; P: Pleomorphism; S: Spindle; Ep: Epithelioid; HPF: High power field
*The cases with insufficient amount and inappropriate quality of DNA extraction
**Cases with unsuccesful PCR amplification in none of the exons
†Cases having KIT gene mutation
‡Cases having PDGFRA gene mutation

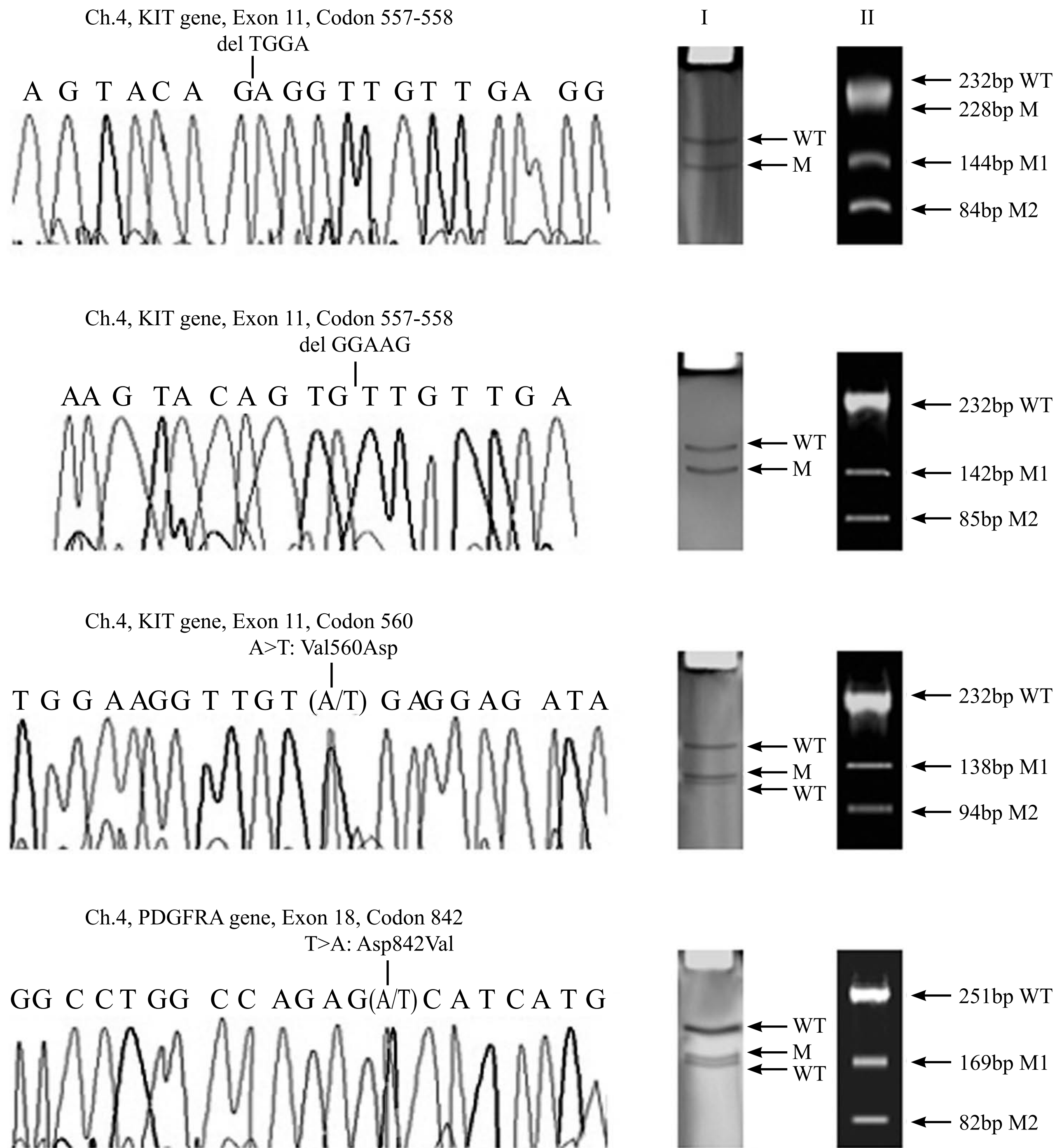

Fig 1. The description of the mutations detected in the series. (I): Comparison of polyacrylamide gel patterns showing conformation diversity of single strand DNA of the cases and their wild type homologous; (II): Agarose gel patterns of the heteroduplexes formed by amplicons and their wild type homologous after 'surveyor' DNA restriction enzyme reaction
A: Adenine; T: Thymine; G: Guanine; C: Cytosine; WT: Wild Type; M: Mutation related fragment; bp: base pair

maining one is single nucleotide alteration type mutation. More than 80% of PDGFRA gene mutations are found to be in exon 18. They are point mutations characterized by replacement of aspartic acid for valine amino acid. Exon 14 and 12 mutations are also commonly missense point mutations. PDGFRA mutation detected in this study is a point mutation characterized by replacement of thymine for adenine in codon 842.

Table 2
The primers designed for target exon amplification of KIT and PDGFRA genes

Target of primer		Primer name and 5’ → 3’ sequence	Amplicon (bp)
KIT	Exon 9	KIT E9-F: TCCTAGAGTAAGCCAGGGCTT KIT E9-R: TGGTAGACAGAGCCTAAACATCC	283
	Exon 11	KIT E11-F: GTGCTCTAATGACTGAGAC KIT E11-R: TACCCAAAAAGGTGACATGG	232
	Exon 13	KIT E13-F: GACATCAGTTTGCCAGTTGT KIT E13-R: TGTTTTGATAACCTGACAGAC	214
	Exon 17	KIT E17-F: ATGGTTTTCTTTTCTCCTCC KIT E17-R: TACATTATGAAAATCACAGG	243
PDGFRA	Exon 12	PDGFRA E12-F: TCCAGTCACTGTGCTGCTTC PDGFRA E12-R: GCAAGGGAAAAGGGAGTCTT	260
	Exon 14	PDGFRA E14-F: TCTGAGAACAGGAAGTTGGTAGC PDGFRA E14-R: CCAGTCAAAATCCTCACTCCA	232
	Exon 18	PDGFRA E18-F: ACCATGGATCAGCCAGTCTT PDGFRA E18-R: TGAAGGAGGATGAGCCTGACC	208

KIT exon 11 mutation is reported to be more commonly seen in GISTs having large size, high mitotic activity and malignant biologic behavior (14-16). Duplicative mutations in exon 11 are found to be more common in gastric GISTs and these tumors have spindle cell phenotype (17,18). Gastric GISTs having exon 13 mutation have worse prognosis (4, 19-24).

PDGFRA mutation is closely related with gastric GISTs. This mutation has been detected in more than 95% of gastric GIST. In such cases, epithelioid or mixed phenotype is usually seen and they have better prognosis (4,25). Many studies revealing the close relation of PDGFRA mutation with epithelioid morphology and gastric localization have been reported (22,24,26). Most of the cases having mutant KIT proteins are sensitive to imatinib treatment. However GISTs having exon 17 mutation are resistant to treatment. GISTs with exon 9 mutation are less responsive to treatment than those with exon 11 mutation (3,27). GISTs with PDGFRA exon 18 mutation are resistant to imatinib treatment (3,28). Because of the limited number of the mutation detected cases in this study, the relationship between morphologic features, biologic

Table 3
The details of PCR method applied for target exon amplification of KIT and PDGFRA genes

PCR tube content	For all exons	
Master mix*	25 µL	
Forward Primer	200 µM	
Reverse Primer	200 µM	
Template DNA	1–100 ng	
Distilled water	Complementary to total volume	
Total volume (µL)	50	
Thermal Cycle Steps		
Activation	5` in 95°C	
Denaturation	40`` in 94°C	x35
Annealing	40`` in 60°C KIT exon 9 and 17 PDGFRA exon 18 40``in 58°C KIT exon 11 and 13 PDGFRA exon 12 and 14	x35
Extension	30``in 72°C	x35
Final extension	5``in 72°C	

*HotStarTaq Plus Master Mix Kit, QIAGEN, Germany

behavior, treatment response and mutational details was not possible to evaluate.

In conclusion, most common genetic alterations seen in GISTs are on the KIT and PDGFRA genes and detection of the mutations in these genes is not only related to morphologic features, metastatic potential and prognosis but also important in determining the specific treatment protocol (29). Therefore, mutation analysis of KIT exons 9, 11, 13,17 and PDGFRA exons 12, 14, 18 should be performed in GISTs after the histopathological diagnosis. For this purpose, a method also has been proposed in this study.

References

1. Gomes AL, Bardales RH, Milanezi F, et al. Molecular Analysis of c-Kit and PDGFRA in GISTs Diagnosed by EUS. Am J Clin Pathol 2007;127:89-96.
2. Joensuu H, Kindblom LG. Gastrointestinal stromal tumors: a review. Acta Orthop Scand Suppl 2004;75:62-71.
3. Miettinen M, Lasota J. Gastrointestinal Stromal Tumors Review on Morphology, Molecular Pathology, Prognosis, and Differential Diagnosis. Arch Pathol Lab Med 2006;130:1466-78.
4. Lasota J, Miettinen M. Clinical significance of oncogenic KIT and PDGFRA mutations in gastrointestinal stromal tumours. Histopathology 2008;53:245-66.
5. Yang J, Du X, Lazar AJF, et al. Genetic Aberrations of Gastrointestinal Stromal Tumors. Cancer 2008;113:1532-43.
6. Straus WM. Preparation of Genomic DNA from mammalian tissue. In: Current protocols in molecular biology. Vol 1. USA: John Willey&Sons Inc.; 2008: Unit 2.2.1-2.2.2.
7. González M, González D, López-Pérez R, et al. Heteroduplex analysis of VDJ amplified segments from rearranged IgH genes for clonality assessments in B-cell non-Hodgkin's lymphoma. A comparison between different strategies. Haematologica 1999;84:779-84.
8. Orita M, Iwahana H, Kanazawa H, et al. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc Natl Acad Sci USA 1989;86:2766-70.
9. Kitamura Y. Gastrointestinal stromal tumors: past, present, and future. J Gastroenterol 2008;43:499-508.
10. He HY, Xiang YN, Li Y, et al. c-kit and PDGFRA mutations in 60 cases of gastrointestinal stromal tumors (GISTs). Beijing Da Xue Xue Bao 2005 18;37:320-4.
11. Zheng S, Chen LR, Wang HJ, et al. Analysis of mutation and expression of c-kit and PDGFR-alpha gene in gastrointestinal stromal tumor. Hepatogastroenterology 2007;54:2285-90.
12. Heinrich MC, Corless CL, Duensing A, et al. PDGFRA activating mutations in gastrointestinal stromal tumors. Science 2003;299:708-10.
13. Lasota J, Stachura J, Miettinen M. GISTs with PDGFRA exon 14 mutations represent subset of clinically favorable gastric tumors with epithelioid morphology. Lab Invest 2006;86:94-100.
14. Lasota J, Jasinski M, Sarlomo-Rikala M, et al. Mutations in exon 11 of c-kit occur preferentially in malignant versus benign gastrointestinal stromal tumors and do not occur in leiomyomas or leiomyosarcomas. Am J Pathol 1999;154:53-60.
15. Taniguchi M, Nishida T, Hirota S, et al. Effect of c-kit mutation on prognosis of gastrointestinal stromal tumors. Cancer Res1999;59:4297-300.
16. Ernst SI, Hubbs AE, Przygodzki RM, et al. KIT mutation portends poor prognosis in gastrointestinal stromal / smooth muscle tumors. Lab Invest 1998;78:1633-6.
17. Kontogianni-Katsarou K, Dimitriadis E, Lariou C, et al. KIT exon 11 codon 557/558 deletion/ insertion mutations define a subset of gastrointestinal stromal tumors with malignant potential. World J Gastroenterol 2008;14:1891-7.
18. Rubin BP. Gastrointestinal stromal tumours: an update. Histopathology 2006;48:83-96.
19. Lasota J, Dansonka-Mieszkowska A, Sobin LH, et al. A great majority of GISTs with PDGFRA mutations represent gastric tumors of low or no malignant potential. Lab Invest 2004;84:874-83.
20. Corless CL, McGreevey L, Town A, et al. KIT gene deletions at the intron 10-exon 11 boundary in GI stromal tumors. J Mol Diagn 2004;6:366-70.
21. 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 Oncol 2002;20:3898-905.
22. Penzel R, Aulmann S, Moock M, et al. The location of KIT and PDGFR-a gene mutations in gastrointestinal stromal tumours is site and phenotype associated. J Clin Pathol 2005;58:634-9.

23. Lasota J, Miettinen M. KIT and PDGFR-a mutations in gastrointestinal stromal tumors (GISTs). Semin Diagn Pathol 2006;23:91-102.
24. Lasota J, Corless CL, Heinrich MC, et al. Clinicopathologic profile of gastrointestinal stromal tumors (GISTs) with primary KIT exon 13 or exon 17 mutations: a multicenter study on 54 cases. Mod Pathol 2008;21:476-84.
25. Wardelmann E, Pauls K, Merkelbach-Bruse S, et al. Gastrointestinal stromal tumors carrying PDGFR-a mutations occur preferentially in the stomach and exhibit an epithelioid or mixed phenotype. Verh Dtsch Ges Pathol 2004;88:174-83.
26. Wasag B, Debiec-Rychter M, Pauwels P, et al. Differential expression of KIT/PDGFR-a mutant isoforms in epithelioid and mixed variants of gastrointestinal stromal tumors depends predominantly on the tumor site. Mod Pathol 2004;17:889-94.
27. Heinrich MC, Corless CL, Demetri CG, et al. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol 2003;21:4342-9.
28. Corless CL, Schroeder A, Griffith D, et al. PDGFRA mutations in gastrointestinal stromal tumors: frequency, spectrum and in vitro sensitivity to imatinib. J Clin Oncol 2005;23:5357-64.
29. Sihto H, Franssila K, Tanner M, et al. Platelet-derived growth factor receptor family mutations in gastrointestinal stromal tumours. Scand J Gastroenterol. 2006;41:805-11.