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Expression of DOG1, PDGFRA, and p16 in Gastrointestinal Stromal Tumors

Sung Hee Jung*, Kwang Sun Suh[†,‡], Dae Young Kang[†], Dong Wook Kang[§], Young-Beum Kim[†], and Eun-Sun Kim[†]

*Department of Internal Medicine, Eulji University Hospital, Eulji University College of Medicine, [†]Department of Pathology and [‡]Research Institute for Medical Sciences, Chungnam National University School of Medicine, and [§]Department of Pathology, Eulji University Hospital, Eulji University College of Medicine, Daejeon, Korea

Background/Aims: The diagnosis of gastrointestinal stromal tumors (GIST) relies on the demonstration of KIT expression, but KIT expression is absent or reduced in approximately 15% of GIST. **Methods:** Eighty-one GISTs were diagnosed between January 1998 and December 2007 at the Department of Pathology at both Chungnam National University Hospital and Eulji University Hospital, Daejeon. Medical history, patient follow-up, and radiographic data were collected if available in the medical records. To determine diagnostic and prognostic markers for GISTs focused on PDGFRA mutation and clinicopathologic features, we analyzed 81 GIST cases for KIT, PDGFRA, DOG1, and p16 expression and for mutation of *PDGFRA* genes. **Results:** Among 81 GIST cases, 20 high risk cases (24.7%) were recurred or metastasized. Immunohistochemically, KIT was positive in 76 (93.8%), PDGFRA in 75 (92.7%), and DOG1 in 77 (95.1%). With a cutoff value of 50%, p16 expression was positive in 26 cases were positive (32.1%). A correlation between p16 expression or negative DOG1 expression and recurrence or metastasis was demonstrated ($p<0.05$). Four cases showed a missense mutation in exon 12 of *PDGFRA* gene, three of these were of epithelioid GISTs. Two cases showed a silent mutation in exon 18 of PDGFRA. **Conclusions:** These results indicate that the expression of DOG1 and PDGFRA is observed in a majority of GIST cases. Expression of p16 and negative DOG1 expression is predictive for development of recurrence and/or metastasis. Even though mutation of the *PDGFRA* gene is frequently seen in epithelioid GISTs, a clinicopathologic correlation was not demonstrated. **(Gut Liver 2011;5:171-180)**

Key Words: Gastrointestinal stromal tumor; Platelet-derived growth factor alpha; Mutation; DOG1; p16

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) originate from the interstitial cells of Cajal (ICC).[1,2] The observation that ICC can be immunohistochemically highlighted with an antibody to KIT (CD117) lead to the discovery that KIT is also strongly expressed in most GISTs.[1-3] Although approximately 95% of GISTs stain positive for KIT (CD117),[3,4] recent molecular studies have determined that some of these tumors are KIT negative.[4,5]

Approximately 80% to 85% of GISTs exhibit activating mutations of KIT tyrosine kinase. Some of these tumors have mutations in the KIT-related kinase gene *PDGF* receptor alpha (*PDGFRA*) in exons 18 (5.6%) or 12 (1.5%). The remainder of GISTs (12%) are wild type (WT) for both *KIT* and *PDGFRA*.[4] The responsiveness of GISTs to treatment using the kinase inhibitor imatinib varies, depending on the exonic location of the *Kit* or *PDGFA* mutation. Corless *et al*.[4] proposed a molecular-based classification of GIST.

KIT-low/negative GISTs are a heterogeneous group comprised, in part, by tumors with *PDGFRA* mutations and, in part, by tumors with *KIT* mutations. The vast majority of *PDGFRA*-mutant GISTs express little or no *KIT*, perhaps because down-regulation of the wild-type *KIT* gene is advantageous.[6]

Most GISTs are comprised of a fairly uniform population of spindle cells (70% of cases), but some are dominated by epithelioid cells (20% of cases), and the remainder consists of a mixture of these two types. Success in treating GISTs with imatinib has emphasized early diagnosis. Fibromatosis and leiomyosarcoma are perhaps the most common tumors misdiagnosed as GIST.[4,7]

Recently, West *et al*.[8] identified DOG1 (TMEM16A) as a gene with a high level of GIST expression and developed a rabbit polyclonal antibody and an *in situ* hybridization probe that target DOG1 using gene expression profiling. A following study showed that mouse monoclonal antibodies against the DOG1

Correspondence to: Sung Hee Jung
Division of Gastroenterology, Department of Internal Medicine, Eulji University Hospital, 1306 Dunsan 2-dong, Seo-gu, Daejeon 302-799, Korea
Tel: +82-42-611-3055, Fax: +82-42-611-3053, E-mail: jsh@eulji.ac.kr
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antibody are more sensitive and more specific than the anti-CD117 reagent.[9]

p16 is a tumor suppressor protein that inhibits cell cycling by arresting cells in G1 before entry into the S phase.[10] Loss of the p16 protein has been reported to be correlated with high-risk GIST and is a predictor of a poor clinical outcome in a variety of human tumors.[11-13] In contrast, an adverse effect of p16 expression on prognosis was recently described.[10,14] The prognostic significance of p16 gene alterations in GIST is still unknown.

In this study, we evaluated the diagnostic and prognostic markers for GIST focused on *PDGFRA* mutations and clinicopathologic features.

MATERIALS AND METHODS

Eighty-one GISTs were diagnosed between January 1998 and December 2007 at the Department of Pathology at both Chungnam National University Hospital and Eulji University Hospital. The studies described here were performed with the approval of the Institutional Review Board at Chungnam National University School of Medicine. Medical history, patient follow-up, and radiographic data were collected, if available, from the medical records.

1. Histologic evaluation

Hematoxylin and eosin (H&E)-stained sections were reviewed by two pathologists in each case. A diagnosis of GIST was made based on tumor location, morphology, and immunostaining for KIT. Five KIT-negative cases were accepted as GIST based on no histologic or immunophenotypic support for smooth muscle differentiation. Mitoses were counted in 50 consecutive high-power fields (HPFs) from the most cellular and mitotically active area. According to tumor size and mitotic activity, GISTs was classified into very low risk, low risk, intermediate risk, and high risk categories according to Fletcher *et al*.[7] The cell type feature was classified as spindle, epithelioid, or mixed cell type (Fig. 1).

2. Immunohistochemical study

Representative areas from 81 GISTs were selected for construction of tissue microarrays using a 3 mm punch. Two punches per case were taken from 81 cases. Immunohistochemical analyses for p16, KIT, PDGFRA, and DOG1 were performed. Four micrometer sections were cut from the tissue microarray blocks and placed onto coated slides. Immunohistochemical staining was performed using a polyclonal anti-CD117 (KIT) antibody (dilution 1:300; Dako, Capinteria, CA, USA), PDGFRA

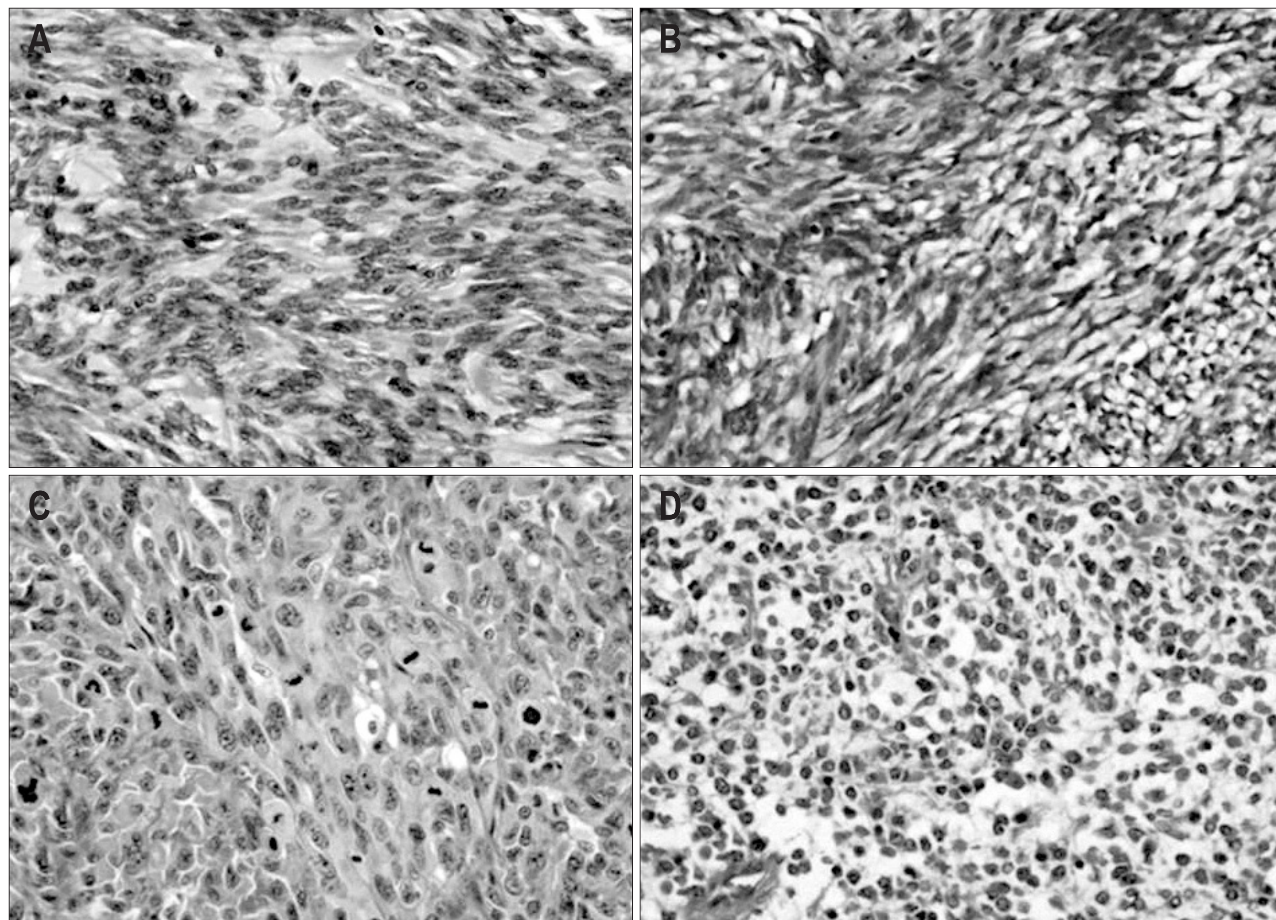

Fig. 1. Gastrointestinal stromal tumors, high-grade. Spindle cell type (A, B: H&E stain, ×400) and epithelioid type (C, D: H&E stain, ×400).

(dilution 1:250; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), and p16 (dilution 1:80), and DOG1 (1:120). Immunohistochemical staining was evaluated via estimation of 10 HPTs.[12] A membranous and/or cytoplasmic staining pattern for KIT, PDGFRA, and DOG1 was considered positive. Nuclear staining with or without a cytoplasmic reaction for p16 was counted. Tissue cores were scored (on the basis of the percentage of positive tumor cells staining above the background) as negative (0%), weakly positive (<10%), moderately positive (10-50%) or diffusely positive (>50%). Scoring results were catagorized as either negative (score of 0 or 1) or positive (score of 2 or 3).[10] For p16, we also analyzed a cutoff value of 50%.[14]

3. DNA extraction

Sixty tumor samples (25 intermediate risk tumors and 35 high risk tumors) were taken from formalin fixed, paraffin embedded (FFPE) tissue samples. H&E-stained 4 μm sections were reviewed under a microscope and areas rich in tumor cells were marked. Corresponding areas on unstained sections were scraped from the slides using a scalpel blade. Tumor samples that contained as few non-neoplastic cells as possible (70-90% tumor cellularity) were collected. A total of 3 to 5 dissected 10 μm sections were incubated at 55°C for one day in 400 μL of DNA extraction buffer (0.25 μg/μL of proteinase K (Roche, Mannheim, Germany), 20 mM Tris/HCl, pH 8.3 mM $MgCl_2$, 100 mM KCl, 1% Tween-20, and 1% NP-40). The mixture was boiled for 10 minutes to inactivate the proteinase K, followed by phenol-chloroform extraction for purification, and then concentrated using ethanol precipitation.

4. PCR amplification of the *PDGFRA* gene

Polymerase chain reaction (PCR) primers were designed to amplify exons 12 and 18 of the *PDGFRA* gene.[15] PCR amplification was performed in a total volume of 20 μL containing 500 ng of template DNA, one unit of ExTaq polymerase (Takara, Shiga, Japan), 1.25 mM dNTP, 15 pmole of primers, and 2 μL of 1 X reaction buffer. PCR cycles consisted of 5 minutes at 94°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 55°C, and 30 seconds at 72°C, followed by one cycle for 7 minutes at 72°C.

5. SSCP analysis, silver staining, and direct sequencing

Two μL of PCR product was mixed with 6 μL of sample loading buffer containing 95% formide (deionized), 10 mM NaOH, 0.25% Bromophenol blue, and 0.25% Xylene cyanol, denatured for 3 minutes at 100°C, and quickly chilled on ice. The solution was then loaded onto 12% polyacrylamide gel containing 1 X sample buffer (33 mM Tris-sulfate, 7% Glycerol, pH 8.3), and electrophoresed at 250 V. After electrophoresis the gel was disassembled from the glass plate, then stained using a Silver Stain Plus kit (Bio-Rad, Hercules, CA, USA), followed by air drying. Samples with abnormal bands were sequenced automatically using a Genetic analyzer (Applied Biosystems, Foster City, CA, USA).

6. Statistical analysis

Statistical analysis was performed using SPSS software (PASW Statistics version 18.0; SPSS Inc., Chicago, IL, USA). Both age and tumor size among the risk groups were analyzed using the Kruskal-Wallis test. Histologic parameters and recurrence were correlated with p16, DOG1, and KIT immunostaining results using a two-sided χ^2 test or Fisher's exact test. A two-tailed value $p<0.05$ was considered to be statistically significant.

RESULTS

1. Clinical presentation

Eighty-one patients (mean age, 58.7 years; SD, 13.5; male/female ratio, 1:0.95; 39 men, 37 women) underwent surgical resection for GIST. Three GIST patients had additional malignant tumors (one stomach cancer, one breast cancer, and one cholangiocarcinoma). Among 81 GISTs, 44 cases (54.3%) occurred

Table 1. Clinicopathologic Features of GISTs

Clinicopathologic features	No. (%)
Localization of primary tumor	
Stomach	44 (54.3)
Small intestine	23 (28.4)
Colon	4 (4.9)
Esophagus	2 (2.5)
Others (EGIST)	8 (9.9)
Histologic pattern	
Spindle cell-like	62 (76.5)
Epithelioid	10 (12.3)
Mixed pattern	9 (11.1)
Risk of malignancy	
Very low risk	5 (6.2)
Low risk	16 (19.8)
Intermediate risk	25 (30.9)
High risk	35 (43.2)
Immunohistochemistry	
c-Kit+	76 (93.8)
p16+ cutoff >10%	36 (44.4)
+ cutoff >50%	26 (32.1)
PDGFRA+	75 (92.7)
DOG1+	77 (95.1)
Recurrence or metastasis	20 (24.7)
PDGFRA mutation (exon)	5
Missense mutation (12-1)	4
Silent mutation (18)	1

GISTs, gastrointestinal stromal tumors.

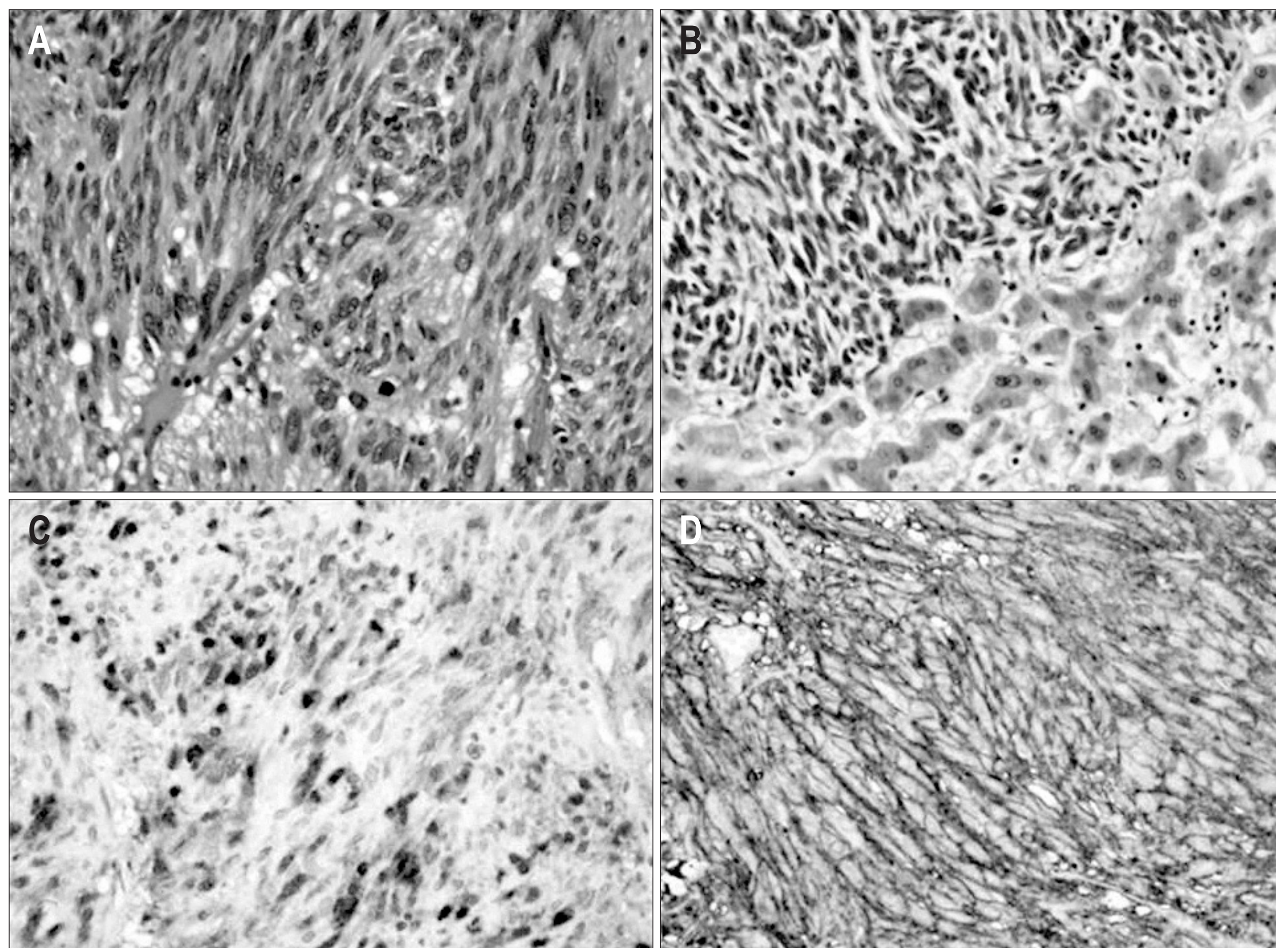

Fig. 2. Gastrointestinal stromal tumors of the small intestine, high-grade, spindle cell type (A, H&E stain, ×400), with liver metastasis within 24 months (B, H&E stain, ×400). Nuclear expression of p16 (C, immunohistochemical stain for p16, ×400) and cell membrane/cytoplasmic staining of DOG1 (D, immunohistochemical stain for DOG1, ×400).

in the stomach, 23 cases (28.4%) in the small intestine, 4 cases (4.9%) in the colon, 2 cases (2.5%) in the esophagus, and 8 cases (9.9%) in the extraintestinal location. A total of 20 (24.7%) high risk GIST patients were affected by tumor recurrence and/or metastases within a median time of 24.3 months (range, 3 to 84 months) (Table 1). Among the high risk group, six patients were treated with imatinib in addition to surgery. Eight patients died of GIST.

2. Histopathologic and immunohistochemical analyses

Sixty two GISTs (76.5%) were predominantly spindle cell type, 10 (12.3%) were epithelioid-like, and 9 tumors (11.1%) exhibited a mixed pattern. The tumors were classified into very low risk (5 cases, 6.2%), low risk (16 cases, 19.8%), intermediate risk (25 cases, 30.9%), and high risk categories (35 cases, 43.2%) (Table 1).[7] The tumor size ranged from 0.3 cm to 24 cm (mean of very low risk group was 1.1 cm; of low risk group was 4.3 cm; of intermediate risk group was 6.6 cm; of high risk group was 11.7 cm). Immunohistochemically, c-kit was positive in 76 (93.8%) of 81 GIST cases, PDGFRA in 75 cases (92.7%), and DOG1 in 77 cases (95.1%). With a cutoff value of 10%, p16 expression was positive in 36 cases (44.4%) and with a cutoff value of 50%, 26 cases were positive (32.1%) (Table 1, Figs. 2 and 3). Among 5 c-kit negative cases, four were DOG+/PDGFRA+ and one was DOG+/PDGFRA- (Table 2). There were no correlations between p16, KIT, DOG1 or PDGFRA expression and the risk of malignancy ($p>0.05$) (Table 3). However, a correlation between p16 expression and recurrence and/or metastasis was demonstrated ($p<0.05$). Negative DOG1 expression was correlated with recurrence and/or metastasis ($p<0.05$) (Table 4).

Table 2. DOG1 and PDGFRA Expression in Five KIT-Negative GISTs

GIST antibody	Very low risk	Low risk	Intermediate risk	High risk
DOG1+/PDGFRA+	5	2	1	1
DOG1+/PDGFRA-	0	1	0	0

GISTs, gastrointestinal stromal tumors.

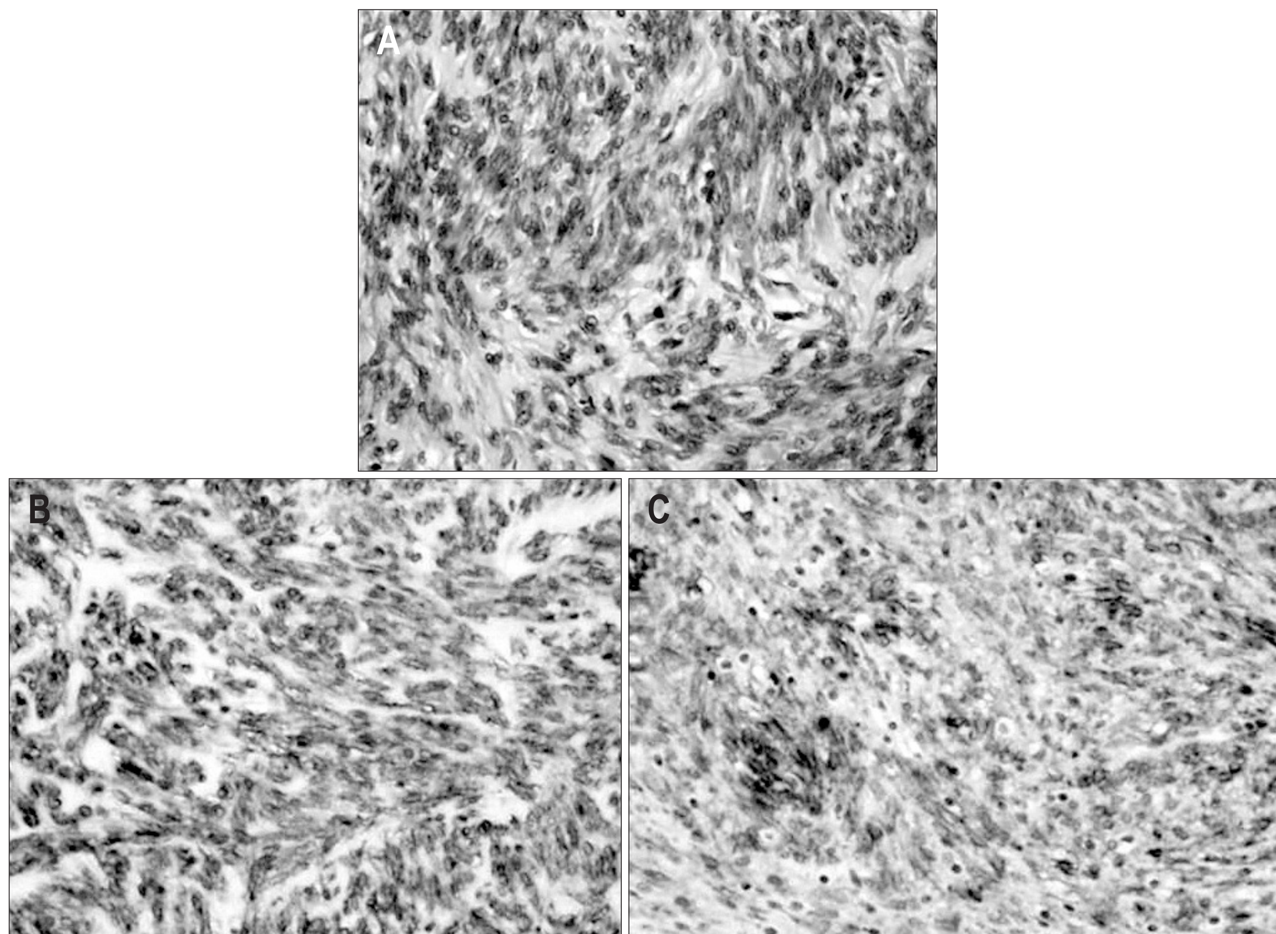

Fig. 3. Gastrointestinal stromal tumors of the stomach, high-grade, spindle cell type (A, H&E stain, ×400), with liver metastasis within 34 months. Cytoplasmic expression of DOG1 (B, immunohistochemical stain for DOG1, ×400) and PDGFRA (C, immunohistochemical stain for PDGFRA, ×400).

Table 3. Clinicopathologic and Immunohistochemical Features of GISTs

		Very low risk (n=5)	Low risk (n=16)	Intermediate risk (n=25)	High risk (n=35)	p-value
						Kruskall-Wallis test
Age, Mean±SD, yr		54.2±12.4	64.5±9.6	58.7±12.4	55.8±11.7	0.1260
Tumor size, Mean±SD, cm		1.1±0.7	4.3±0.8	6.6±2.2	11.7±7.7	<0.0001*
p16						Chi-square test
Cut off >10%	+	2	6	8	20	0.2400
	-	3	10	17	15	
Cut off >50%	+	1	5	6	14	0.5527
	-	4	11	19	21	
c-kit	+	5	13	24	34	0.1347
	-	0	3	1	1	
PDGFRA	+	5	14	22	34	0.4185
	-	0	2	3	1	
DOG1	+	5	16	25	31	0.1368
	-	0	0	0	4	
Recurrence	+	0	0	0	20	<0.0001*
	-	5	16	25	15	

GISTs, gastrointestinal stromal tumors.
*p-value.

Table 4. Pathologic and Immunohistochemical Features of GISTs with or without Recurrence or Metastases

Parameters		Recurred case (%) (n=20)	No recurrence (%) (n=61)	p-value
Histologic grade				Chi-square test
Very low risk		0 (0)	5 (6.2)	<0.0001*
Low risk		0 (0)	16 (19.8)	
Intermediate risk		0 (0)	25 (30.9)	
High risk		20 (24.7)	15 (18.5)	
				Fisher's exact test
c-Kit	+	19 (23.5)	57 (70.4)	1.0000
	-	1 (1.2)	4 (4.9)	
PDGFRA	+	19 (23.5)	56 (69.1)	1.0000
	-	1 (1.2)	5 (6.2)	
p16				
Cutoff >10%	+	13 (16.0)	23 (28.4)	0.0407*
	-	7 (8.6)	38 (46.9)	
Cutoff >50%	+	12 (14.8)	14 (17.3)	0.0047*
	-	8 (9.8)	47 (58.0)	
DOG1	+	16 (19.8)	61 (75.3)	0.0029*
	-	4 (4.9)	0 (0)	

GISTs, gastrointestinal stromal tumors.
*p-value.

3. *PDGFRA* mutations

Three cases showed a missense mutation in exon 12-1 of *PDGFRA* and one case showed a missense mutation in exon 12-2 of *PDGFRA*. Three of four exon 12 mutated GISTs were epithelioid (Figs. 4 and 5). The remaining one was mixed cell type (Table 5). Two *PDGFRA*-mutated GISTs developed in the stomach, one was from the colon, and one was from the esophagus. Among four exon 12 mutated GISTs, 3 were high risk and 1 was intermediate risk. One epithelioid type also showed a silent mutation in exon 12-1. One mixed cell type and one spindle GIST also showed a silent mutation in exon 18 of *PDGFRA*. All *PDGFRA*-mutated GISTs were positive for KIT, PDGFRA, or DOG1 except one case. No significant clinicopathologic correlation between PDGFRA expression and mutation was demonstrated. There was no correlation between *PDGFRA* mutation and recurrence and/or metastasis.

DISCUSSION

Most (~95%) GISTs show positive immunoreactivity for KIT protein expression.[3,4] However, recent studies have identified a small group of KIT-negative GISTs[4,5] with *KIT* or *PDGFRA* mutations, which may be sensitive to imatinib therapy. These cases require special attention for diagnosis.[4,5] Diagnosis of a KIT-negative GIST can be supported by immunostains for desmin and the S-100 protein, which exclude smooth muscle tumors and neural tumor like schwannomas.[16]

Molecular analysis of the *KIT* and *PDGFRA* genes is necessary

Table 5. Clinicopathologic Characteristics of Patients with *PDGFRA* Gene Mutations

Case no.	Age/Sex	Site	Risk group	Cell type	KIT IHC	PDG-FRA IHC	DOG1 IHC	p16 IHC	*PDGFRA* mutation				Dis.status (F/U mo)
									Exon	Nucleotide change	Amino acid change	Mutation	
10	52/M	Esophagus	High	Epithelioid	+	+	+	-	12-2	TAT→AAT	Tyr→ Asn (Y573N)	Missense mutation	Recurrence (4) Expire (7)
11	69/M	Stomach	High	Spindle	+	+	+	-	18	GGA→GGT	Gly→ Gly (G829G)	Silent mutation	NED (60)
14	63/F	Stomach	Intermediate	Mixed	+	+	+	-	12-1	ATG→AGG	Met→ Arg (M578R)	Missense mutation	NED (24)
									18	GGA→GGT	Gly→ Gly (G829G)	Silent mutation	
28	60/M	Stomach	High	Epithelioid	+	+	+	-	12-1	ATG→AAG	Met→ Lys (M578K)	Missense mutation	Peritoneal seeding (37) Expire (40)
36	40/M	Colon	High	Epithelioid	+	+	-	+	12-1	ATG→AGG	Met→ Arg (M578R)	Missense mutation	Liver metastasis (5)
									12-1	GAC→GAT	Asp→ Asp (577)	Silent mutation	Expire (7)

F/U, follow-up.

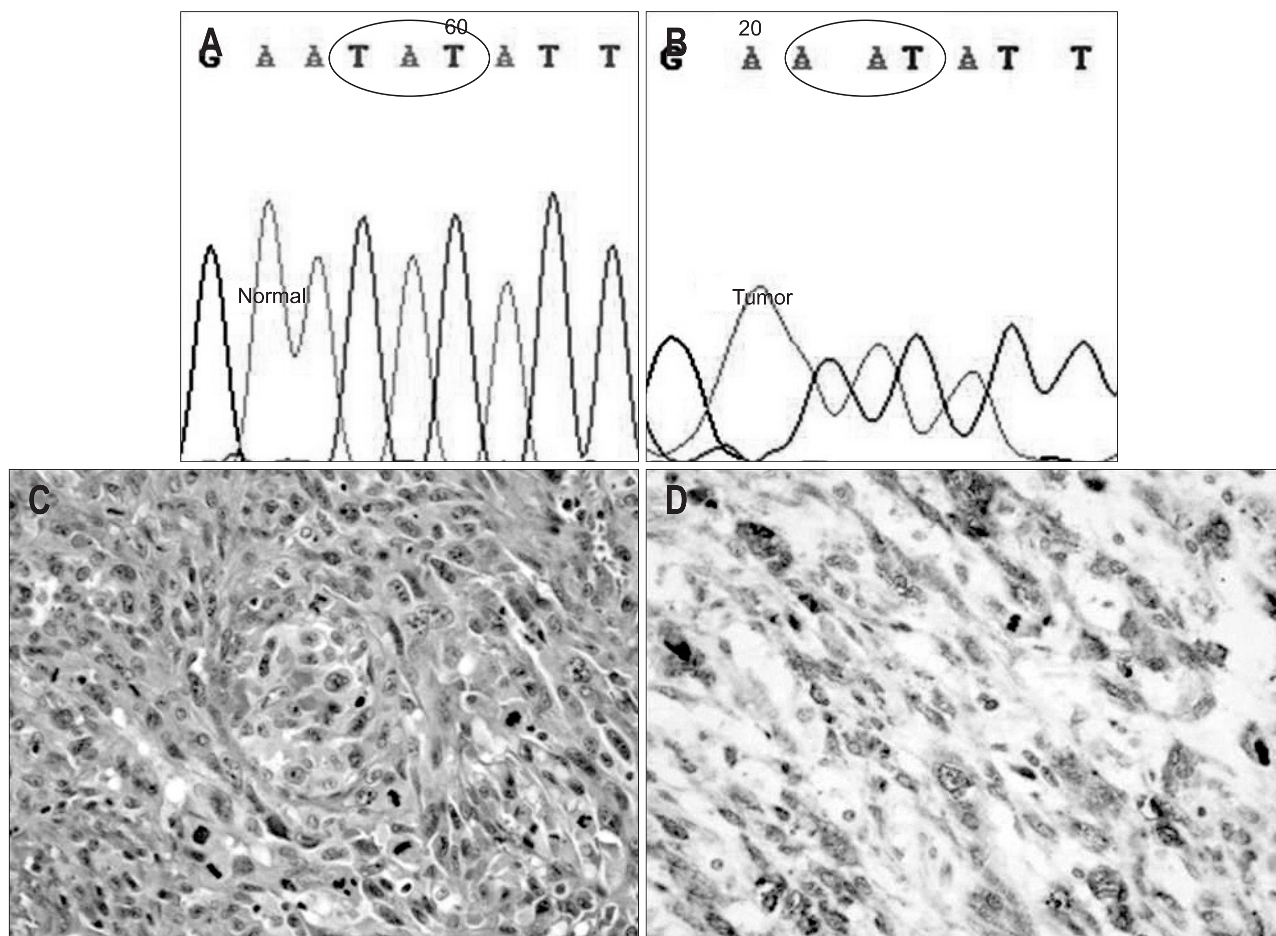

Fig. 4. Demonstration of *PDGFRA* mutations (T deletion) in exon 12-2 (TAT→AAT, Tyr→Asn: Y573N, missense mutation) (A, normal; B, tumor). Epithelioid gastrointestinal stromal tumors of the esophagus, high-grade (C, H&E stain, ×400), showing strong PDGFRA expression (D, immunohistochemical stain for PDGFRA, ×400). This tumor recurred within 4 months following the operation (case no. 10).

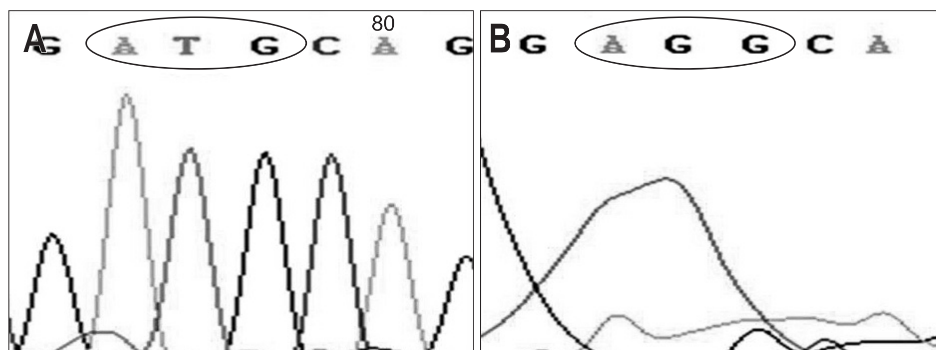

Fig. 5. Demonstration of a *PDGFRA* (T→G) mutation in exon 12-1 (ATG→AGG, Met→Arg (M578R), missense mutation) (A, normal; B, tumor) (case no. 14).

for accurate diagnosis of KIT-immunonegative GISTs, but practical application is difficult in the routine diagnostic process. Therefore, the diagnosis of GIST still depends on immunohistochemical staining. Recent studies have reported that PDGFRA, protein kinase θ (PKCθ), and FLJ10261 (DOG1, discovered on GIST-1) expressions were detected in WT *KIT* GIST. Therefore, PDGFRA, PKCθ, and FLJ10261 can be used as diagnostic markers for GIST, especially in KIT negative cases.[17]

In this study, we found that PDGFRA, like KIT, was expressed in the majority (92.7%) of GISTs. Recently, the routine use of PDGFRA immunophenotyping has been reported to be a useful diagnostic tool, especially in KIT-negative cases, as it correctly predicts the presence of *PDGFRA* mutations.[18] KIT-negative GISTs were positive for PDGFRA and PDGRFA-negative GISTs were positive for KIT (CD117).[17,18] Therefore, both PDGFRA and KIT (CD117) can be used for diagnosis and differential diagnosis of GISTs. According to Zheng *et al*.,[17] PDGFRA protein expression cannot be used as a prognostic index. In our study,

PDGFRA protein expression showed no correlation with clinocopathologic parameters in GIST patients.

Another diagnostic marker has been developed for accurate diagnosis of GISTs. Recently, West *et al*.[8] characterized gene expression patterns in GISTs using a cDNA microarray and found that the gene FLJ10261 (DOG1, discovered on GIST 1), encoding a hypothetical protein, was specifically expressed in GISTs. A new mouse monoclonal antibody against DOG1 was reported to have a high sensitivity and specificity for GISTs.[9] With the use of DOG1.1, more than a third of KIT-negative GISTs can be classified using IHC.[19] DOG1.1 is an especially sensitive immunohistochemical marker for GIST, and has potential for clinical use in the routine diagnosis of GIST.[9] DOG1 has been recently identified as a gene in the *CCND1-EMS1* locus on human chromosome 11q13, which is amplified in several cancers, including head and neck, bladder, and breast.[20] Although DOG1 was found to be expressed in various tumors, the biological function and the overexpression mechanism in GIST are still unknown. West *et al*.[8] suggested two possible mechanisms. ICCs are immunoreactive for DOG1, as in KIT. This finding suggests the possibility that the protein has a role in receptor kinase type III signal transduction pathways. On the other hand, DOG1 may be a fortuitous marker of the GIST phenotype with no direct connection to the KIT and PDGFRA signaling pathways.[12] DOG1 was highly expressed in *KIT*-and *PDGFRA*-mutant GISTs.[8,9] These results have important clinical value in identifying patients for imatinib therapy. Therefore, DOG1 may play a role in development of GIST and may be an additional diagnostic marker and potential therapeutic target in GIST.

There have been several studies indicating that DOG1 may be a new diagnostic marker for GIST, however, its prognostic implication is still unknown. Espinosa *et al*.[9] reported that DOG1.1 expression was not related to the type of mutation (*KIT* or *PDGFRA*), site, tumor size, tumor grade, or patient age. In our study, DOG1 was expressed in 95.1% of cases, and DOG1-negative GIST cases were significantly correlated with recurrence and/or metastasis ($p=0.0029$). These findings indicate that DOG1 is a new diagnostic marker with potential to also be a prognostic marker.

GISTs are characterized by alterations in genes involved in cell cycle regulation. p16 (INK4A) is a tumor suppressor protein that inhibits cell cycling by arresting cells in G1 before entry into the S phase.[14] Although p16 has been extensively investigated in GISTs, there are still discrepancies regarding its prognostic value.[10] Herein, we studied immunohistochemical staining for p16 with >10% and >50% cutoff values to see if it can aid in clinical prognostic assessment in GISTs. In our study, patients expressing p16 were found to do worse than those not expressing p16. GISTs with p16 protein expression had a significantly higher recurrence rate and/or metastatic behavior (>10% cutoff value, $p<0.0407$; >50% cutoff value, $p<0.0047$). Two similar studies of the effect of p16 protein expression on prognosis have recently reported that expression of p16 significantly correlates with a poor prognosis in GIST.[10,14] Schmieder *et al*.[14] reported that in patients with high risk GIST, the immunohistochemical expression of the p16 protein was highly predictive ($p<0.05$) for a poor prognosis (the development of recurrence). They suggested that, in addition, p16 expression might be an indicator for "very high risk GIST." They analyzed prognoses with >10%, >20%, and >50% p16 expression cutoff values. Survival decreased significantly with a cutoff value >50%.

In contrast to our results, loss of the p16 protein was correlated with high risk GIST and poor clinical outcomes in several studies.[11-13] p16 gene alterations correlated significantly with loss of p16 protein expression. p16 protein loss can be caused by many mechanisms, as shown in other studies,[11-13] including loss of heterozygosity of chromosome 9p, methylation of the p16 gene promoter region, a loss-of-function mutation, or a submicroscopic small deletion of the *CDKN2A* gene locus.[11-13] However, which pathophysiological role p16 plays in the oncogenesis of GIST remains unknown and it might even change at different stages of tumorigenesis. Loss of p16 expression contributes to malignancy and genetic alterations, and diminished p16 levels are common in human cancers.[21] However, expression of the p16 protein correlates with an unfavorable prognosis and a poor clinical outcome in GIST.[10,14] This implies that p16 loss is not required for oncogenesis, and other mechanisms upsetting cell-cycle control may be involved.[10]

Although the predictive value of p16 in GIST has been determined, the prognostic significance of p16 gene alterations in GIST is still under debate.[11-13,21] It is difficult to estimate the predictive value of p16 in GIST because different methods, different cutoff values, different follow-up durations, and different search variables have been used and the comparability between studies is limited. Each study used different positive values of p16 expression with different cutoff values.[10-14] In our study, we found that 44.4% of cases were positive for p16 immunostaining with a cutoff value of 10%, and 32.1% were positive with a cutoff value of 50%. With a cutoff value of 10% the reported p16 immunostaining positivity in defined GIST was 42%,[14] and with a cutoff value of 50%[12] the reported positivity was 43%.[10]

KIT and *PDGFRA* genes encode KIT and PDGFRA, which belong to the type III transmembrane tyrosine kinase receptor family.[4] Mutation of *KIT* has been implicated as a major genetic event in the tumorigenesis of GISTs because most GISTs show a gain-of-function mutation in *KIT*.[1] Recently, the mutation of *PDGFRA* has been considered as another causative genetic event[6] as *PDGFRA* mutations were found in most GISTs lacking a *KIT* mutation. Constitutional *KIT* gene mutations were observed in 75% to 80% of GISTs.[4,22,23] *PDGFRA* gene mutations are observed in up to 22.5% of cases.[22-26] *PDGFRA* mutations occur preferentially in exon 18 and rarely in exon 12.[23] *PDGFRA*-mutant tumors arise primarily in the stomach, mesentery, and omentum.[27,28] In this study, we found that *PDGFRA* muta-

tions were identified in 5 (8.3%) of 60 cases (intermediate and high risk groups). These cases showed both KIT and PDGFRA expression. Four GISTs showed a missense mutation of exon 12 (three cases for exon 12-1 and one case for exon 12-2), with two cases showing a silent mutation in exon 18. Mutations involved codons 578 and 753. In four tumors (2125C→A, n=2, or T→A, n=2) missense mutations leading to substitution of lysine for asparagines (Y573N, M578R) were identified. No Y573N, M578K, or M578R mutant, which was identified in our study, was found in the literature. However, it has been reported that all exon 12 *PDGFRA* mutant GISTs were clustered between 560 and 577 PDGFRA amino-acid residues, and that this region should be considered as a minor mutational "hot spot" for GIST.[27] A *PDGFRA* mutation in KIT negative GIST was not confirmed as a *KIT* gene mutation study was not performed in this study.

There is a large variation between the apparent frequencies of *PDGFRA* mutation in different studies. The frequency of *PDGFRA* mutations differs between 0-22.5%.[22-26] In other studies in Korea, *PDGFRA* gene mutations were observed in 3.1%[29] and 13.6%[30] of cases. However, an exon 12 mutation was not found in other studies. Several factors, such as baseline characteristics of the enrolled population, different anatomical sites for enrolled GISTs, different diagnostic criteria, ethnic or racial factors, and technical problems, may have affected these results. We found a significant association between *PDGFRA* mutation and the epithelioid/mixed phenotype. It has previously been observed that the vast majority of *PDGFRA* mutant GIST have been found to be associated with a gastric location and a predominantly epithelioid morphology.[5,27-29] Several recent studies have proposed that the type and location of *PDGFRA* mutations in GIST can be used to predict the response to imatinib treatment. The most common *PDGFRA* mutation, D842V in exon 18, is resistant to imatinib.[6,25,27] In contrast, the substitution V561D in exon 12 results in an isoform of *PDGFRA* that is highly sensitive to imatinib.[6,27] Lasota *et al*.[27] and Heinrich *et al*.[6] reported imatinib sensitivities for a deletion/substitution (SPDGHE566-571R) and an in-frame insertion mutation (ER561-562) in exon 12. In our study, four patients with *PDGFRA* exon 12 mutated GIST did not undergo imatinib treatment, so we could not determine the response to imatinib treatment.

Previous studies showed a tendency of a better prognosis for *PDGFRA* than for *KIT* mutated tumors.[26,27] In contrast, our study identified 3 cases of either a short survival or an unfavorable outcome associated with an exon 12 GIST mutation, all with an epithelioid morphology and a high grade malignancy. However, the number of *PDGFRA*-mutant GISTs reported in our study was relatively small, so an unfavorable prognosis for *PDGFRA* mutant GIST could not be confirmed.

In summary, expression of DOG1 and PDGFRA is observed in a majority of GISTs. Expression of p16 and negative DOG1 expression is predictive for development of recurrence and/or metastasis. Even though mutation of the *PDGFRA* gene is frequently seen in epithelioid GISTs, the significance of a clinicopathologic correlation between PDGFRA expression and mutation was not demonstrated. PDGFRA and the DOG1 immunostaining can be useful in diagnosis and differential diagnosis of GISTs. DOG1 has potential to be both a diagnostic marker and a prognostic marker. GISTs with p16 protein expression have a significantly higher recurrence rate; however, the prognostic significance in GIST is still unknown.

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