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Characteristics of KIT-negative gastrointestinal stromal tumours and diagnostic utility of protein kinase C theta immunostaining

H E Lee,¹ M A Kim,¹ H S Lee,³ B L Lee,² W H Kim^{1,2}

¹ Department of Pathology, Seoul National University College of Medicine, Seoul, Korea; ² Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea; ³ Department of Pathology, Seoul National University Bundang Hospital, Seongnam, Korea

Correspondence to: Dr W H Kim, 28 Yeongseon-dong, Seoul 110-799, Korea; woohokim@snu.ac.kr

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ABSTRACT

Aims: To characterise KIT-negative gastrointestinal stromal tumours (GISTs) clinically, pathologically, immunohistochemically and genetically, and to establish the usefulness of protein kinase C theta (PKCθ) as a diagnostic marker in KIT-negative GIST.

Methods: 252 consecutive cases of GIST were evaluated for clinicopathological characteristics and immunostained for various antibodies. Mutational analyses of *KIT* and platelet-derived growth factor receptor α (*PDGFRA*) were also performed in 62 cases.

Results: 20 (7.9%) GISTs showed negative immunostaining for KIT. KIT-negative GISTs were more likely to originate from omentum or peritoneum, have an epithelioid histology, and be classified as high risk. The overall survival rate of patients with KIT-negative GISTs (5-year survival rate 68.7% (SD 10.7%)) was lower than that of patients with KIT-positive GISTs (5-year survival rate, 79.9% (3.0%)) ($p = 0.042$, log-rank test). Negative KIT expression was an independent prognostic factor in multivariate Cox regression analysis when the risk of aggressive behaviour and the status of imatinib treatment were adopted as covariates. KIT-negative GISTs also showed lower expression rates of CD34, Bcl-2, and PKCθ than KIT-positive GISTs; mutational analysis revealed that 30% of KIT-negative GISTs harboured a *PDGFRA* exon 18 mutation. Immunostaining on PKCθ showed that 93.9% of all GISTs expressed PKCθ protein. However, 21.9% of 64 mesenchymal tumours other than GIST also showed positivity on PKCθ.

Conclusions: KIT-negative GISTs had characteristics that differ from those of KIT-positive GISTs, and negative KIT expression was an independent prognostic indicator for overall survival of patients. Although PKCθ is a sensitive diagnostic marker for GIST, its usefulness is limited because of low sensitivity and low specificity in KIT-negative GISTs.

Gastrointestinal stromal tumour (GIST) is the most common mesenchymal tumour of the gastrointestinal tract, and is thought to originate from interstitial cells of Cajal.¹ GIST is characterised by the expression of the type III receptor tyrosine kinase KIT encoded by the *KIT* proto-oncogene,² thus KIT immunohistochemistry (IHC) has been used as a diagnostic marker of GIST.^{2–3} Since Hirota *et al* first reported the *KIT* mutation in 1998,⁴ many reports have demonstrated that the majority of GISTs harbour oncogenic mutations of *KIT*.^{5–6} It was also discovered that a small subset of GISTs (5–7%) has activating mutations of platelet-derived growth factor receptor α (*PDGFRA*).^{7–8} Recently, therapeutic targeting of mutant KIT with the

tyrosine kinase inhibitor, imatinib mesylate has proven to be effective in patients with advanced or unresectable GISTs.^{9–10}

Previous studies have also indicated that 5–10% of GISTs express little or no KIT.^{11–12} These KIT-negative GISTs are believed to be somewhat different from KIT-positive GISTs clinically, pathologically and genetically. Medeiros *et al* reported that KIT-negative GISTs were more likely to have epithelioid cell morphology, arise in the omentum/peritoneal surface, and harbour oncogenic mutations of *PDGFRA*.¹³ Other papers about KIT-negative GISTs have described similar features.^{14–15} However, there have been few direct comparisons of KIT-negative GISTs with KIT-positive GISTs clinically, pathologically, immunohistochemically and genetically, and few data on disease-free survival (DFS) and overall survival (OS) rate of patients with KIT-negative GISTs.

The observation that a subset of KIT-negative GISTs is also responsive to imatinib emphasised the importance of correct diagnosis in KIT-negative cases.¹⁶ Although GIST diagnosis is based on clinical and histopathological features, it needs experience and can be subjective. Recently, IHC of DOG1 and protein kinase C theta (PKCθ) was suggested as a diagnostic marker of GISTs, especially in KIT-negative cases.^{15–17–20} Gene expression studies using DNA microarrays have also revealed that the gene encoding PKCθ is overexpressed in GISTs.^{21–22} In addition, some previous reports showed that PKCθ is highly expressed in GIST tissues by IHC and suggested that PKCθ immunostaining can be used as a diagnostic marker of GIST.^{15–18–20} However, more data on sensitivity and specificity of PKCθ for GIST diagnosis are required before PKCθ can be used as a diagnostic marker of GIST in routine clinical study.

Here, we compare the clinicopathological, immunohistochemical and genetic characteristics of KIT-positive and KIT-negative GISTs and evaluate the usefulness of PKCθ as a diagnostic marker.

METHODS

Tissue samples

A total of 252 surgically resected consecutive GIST cases examined at the Department of Pathology, Seoul National University Hospital over a period of 16 years (1989 to 2005) were collected. These tumours were clinically and histologically classified as GISTs based on previous experience of the clinical and histological spectrum of GIST. In detail, the tumours showed intersecting fascicles,

Table 1 Antibodies used for the immunohistochemical study

Antibody	Retrieval methods	Dilution	Source
KIT	Microwave	1:250	Dako†
CD34	Microwave	1:100	Immunotech‡
SMA	Microwave	1:150	Dako†
S-100	None	1:150	Dako†
Desmin	Autoclave	1:150	Dako†
Bcl-2	Microwave	1:100	Dako†
PKCθ	Autoclave in Borg Decloaking Chamber (Biocare Medical)*	1:15	BD Biosciences§

*Biocare Medical, Walnut Creek, California, USA; †Dako, Glostrup, Denmark;

‡Immunotech, Marseille, France; §BD Biosciences, Franklin Lakes, New Jersey, USA.

nuclear palisading, organoid pattern, or rarely, less cohesive pattern, being composed of spindle or epithelioid cells with eosinophilic cytoplasm and ill-defined cytoplasmic border. Most of the tumours had uniform appearance with evenly distributed chromatin, but rare tumours showed pleomorphic and anaplastic features. The diagnosis of GIST was confirmed by KIT positivity on IHC. For KIT-negative cases, the diagnosis of GIST was limited to the tumours with negative staining on smooth muscle actin (SMA) and desmin on IHC.

Age and sex of patients and tumour site and size were evaluated by reviewing pathological records and/or medical charts. Glass slides were reviewed to determine histological features. The risk of aggressive behaviour was assessed using the criteria of the National Institute of Health GIST workshop.³ Disease stage at presentation (local, loco-regional and metastatic disease) and resection margin status (R0, complete resection; R1, microscopic incomplete resection; R2, macroscopic incomplete resection) of each case were also obtained. Whereas none of the patients had received preoperative chemotherapy or radiotherapy, 33/249 patients (13.3%) were treated with imatinib due to residual, metastatic or recurrent disease after surgery. Follow-up of patients has been performed

Table 2 Primer sequences used in this study

	Forward	Reverse
<i>KIT</i> exon 9	ATGCCACATCCCAAGTGT	TGACATGGTCAATGTTGAA
<i>KIT</i> exon 11	CGTAATCGTAGCTGGCATGA	AAACAAAGGAAGCCACTGGA
<i>PDGFRA</i> exon 12	TCCAGTCACTGTGCTGCTTC	TCTGCCAAGGCCTATAAATTG
<i>PDGFRA</i> exon 18	ACCATGGATCAGCCAGTCTT	GCACCGAATCTCTAGAAGCAA

with imaging modalities including abdomen CT and/or ultrasonography in all cases; the interval of follow-up was usually 6 month. Disease status was categorised as dead of disease, dead of other cause, alive without disease, alive with disease, or lost during follow-up. The duration of DFS was the time between surgery and first recurrence/metastasis or last follow-up. Recurrence and metastasis were defined when any subsequent occurrence of tumour in and outside the original location of tumour was suspicious on imaging, respectively. The duration of OS was the interval between surgery and death or last follow-up. Deaths from unrelated causes were censored.

Fifty-three cases of surgically resected non-GIST mesenchymal tumours in the gastrointestinal tract (non-GIST) (35 leiomyomas, 7 leiomyosarcomas, 7 schwannomas, 2 desmoid tumours, 1 inflammatory fibroid polyp, and 1 undifferentiated sarcoma) were collected from Seoul National University Hospital and examined for PKCθ expression status using the tissue microarray method. In addition, 11 desmoid tumours from Seoul National University Bundang Hospital were examined and immunostained with PKCθ antibody using full section slides.

Tissue microarray method and immunohistochemistry

Core tissue biopsies (2 mm in diameter) were taken from individual paraffin-embedded tumour tissues and arranged in new recipient paraffin blocks (tissue array block) using a trephine apparatus (Superbiochips Laboratories, Seoul, Korea). Each tissue array block contained up to 60 cases, and the eight

Figure 1 KIT-negative gastrointestinal stromal tumours. (A,B) The case with platelet-derived growth factor receptor α (*PDGFRA*) mutation (D842V). (A) The tumour shows vague organoid pattern and is composed of epithelioid cells with myxoid stroma. (B) The tumour represents total negativity on KIT immunostaining. Interspersed mast cells are positive for KIT. (C,D) The case with no *KIT* or *PDGFRA* mutation. (C) The tumour shows epithelioid cell histology. (D) KIT immunohistochemistry is totally negative. Original magnification, $\times 200$.

Original article

array blocks prepared containing a total of 252 GIST and 53 non-GIST tissues. Sections (4 µm) were cut from each tissue array block, dewaxed, and dehydrated.

Immunohistochemical staining for various proteins was performed on microarray slides using a streptavidin peroxidase procedure. Table 1 present the primary antibodies used in this study. For KIT, tissue sections were microwaved in sodium citrate buffer, pH 6.0 for 15 min for epitope retrieval. For PKCθ, antigen retrieval was performed by autoclaving the tissue sections in Borg Decloaking Chamber (Biocare Medical, Walnut Creek, California, USA) at 120°C for 3 min. The sections were incubated with the antibodies to KIT (1:250, rabbit polyclonal; Dako, Glostrup, Denmark) or PKCθ (1:15, mouse monoclonal, clone 27; BD Biosciences, Franklin Lakes, New Jersey, USA). Antibody binding was detected using an avidin–biotin–peroxidase complex (Vectastatin Elite ABC Kit PK-6100; Vector Laboratories, Burlingame, California, USA) for 30 min. The peroxidase reaction was visualised by incubating with 3,3'-diaminobenzidine tetrahydrochloride (DAB Kit SK-4100; Vector Laboratories) for 3 min. Interspersed mast cells and interstitial cells of Cajal were used as a positive control for KIT. For positive control of PKCθ, nerve plexus was used. Smooth muscle was a negative control for both KIT and PKCθ staining. Staining results were scored as positive (immunoreactivity in ≥10% of tumour cells) or negative (immunoreactivity in <10% of tumour cells).

Specifically, 30 cases showing complete negativity or very weak positivity for KIT immunostaining in microarray slides were subject to repeat IHC using full section slides considering possible heterogeneity of tumour in GIST. Finally, KIT-negative GIST was referred when the tumour showed immunoreactivity in <10% of tumour cells in their full section slide. As a result, 20 cases were categorised as KIT-negative GIST. We also confirmed PKCθ IHC results using full section slides for all 20 KIT-negative GISTs.

DNA extraction and direct sequencing

Genomic DNA of formalin or methanol fixed paraffin embedded tissues, including 62 cases of randomly selected KIT-positive GISTs and all 20 cases of KIT-negative GISTs, was extracted using the standard proteinase-K digestion/phenol-chloroform procedure. PCR was performed using 1 µl DNA solution, 0.1 µl TaKaRa Taq (TaKaRa Bio, Shiga, Japan), 1 µl dNTP (2 mM each), 1 µl buffer, 0.5 µl each primer (5 pmol/µl) in a final volume of 10 µl. Table 2 presents the primer sequences used in this study. Thirty-five amplification cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min were performed in GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California, USA). PCR products were treated with ExoSAP-IT (USB, Cleveland, Ohio, USA) and sequenced using BigDye Terminator v3.1 Ready Reaction Cycle Sequencing Kits (Applied Biosystems). Sequenced products were analysed on an ABI Prism 3100 genetic Analyser (Applied Biosystems).

Statistical analyses

The χ^2 test or Fisher's exact test was used to determine correlations between KIT expressional status and clinicopathological parameters, IHC results or mutational status. Student t test was used to compare means of patient's age, tumour size, and mitotic count of tumours between KIT-positive and KIT-negative GISTs. Survival curves were estimated using the Kaplan–Meier product-limit method; the significance of

difference between survival curves was determined using the log-rank test. Multivariate survival analysis using the Cox proportional hazards model was performed adopting the status of KIT expression, the risk of aggressive behaviour, and the status of imatinib treatment as covariates. Results were considered statistically significant for p values less than 0.05. All statistical analyses were conducted using SPSS V.15.0.

RESULTS

Clinicopathological characteristics of KIT-positive and KIT-negative GISTs

The 252 GIST cases were divided into KIT-positive (n = 232; 92.1%) and KIT-negative GISTs (n = 20; 7.9%) according to IHC results. Table 3 summarises clinicopathological characteristics. Median age of patients of KIT-positive and KIT-negative GIST was 58 years (range 23–85) and 56 years (range 26–82), respectively; median size of tumours in KIT-positive and KIT-negative cases was 6.0 cm (range 0.3–23.0) and 6.0 cm (range 0.5–30.0), respectively. Compared with KIT-positive GISTs, KIT-negative GISTs more often originated from colon, omentum or mesentery. Fifty-five per cent of KIT-negative GISTs had

Table 3 Clinicopathological characteristics of KIT-positive and KIT-negative gastrointestinal stromal tumours

	KIT-positive cases n (%)	KIT-negative cases n (%)	p Value
Mean age (SD) (years)	57.10 (11.36)	56.80 (12.78)	0.909†
Sex			0.302‡
Male	123 (53.0)	13 (65.0)	
Female	109 (47.0)	7 (35.0)	
Primary site			0.042*
Stomach	133 (57.3)	10 (50.0)	
Small bowel	69 (29.7)	3 (15.0)	
Large bowel	14 (6.0)	4 (20.0)§	
Omentum or mesentery	16 (6.9)	3 (15.0)	
Mean of tumour size (SD) (cm)	7.13 (5.20)	10.30 (10.02)	0.177†
Mean of mitotic counts/50 HPF (SD)	14.35 (26.22)	45.20 (76.40)	0.088†
Histological type			<0.001‡
Spindle	198 (86.5)	9 (45.0)	
Epithelioid	31 (13.5)	11 (55.0)	
Risk of aggressive behaviour			0.316*
Very low risk	21 (9.1)	3 (15.0)	
Low risk	55 (23.7)	3 (15.0)	
Intermediate risk	52 (22.4)	2 (10.0)	
High risk	104 (44.8)	12 (60.0)	
Stage at presentation			0.006*
Local disease	203 (88.6)	13 (65.5)	
Loco-regional disease	2 (0.9)	0 (0)	
Metastatic disease	24 (10.5)	7 (35.5)	
Resection margin status			0.791*
R0	193 (84.3)	17 (85)	
R1	5 (2.2)	0 (0)	
R2	31 (13.5)	3 (15)	
Last follow-up status			0.064*
Dead of disease	43 (18.5)	6 (30)	
Dead of other cause	12 (5.2)	3 (15)	
Alive without disease	92 (39.7)	6 (30)	
Alive with disease	11 (4.7)	1 (5)	
Lost	74 (31.9)	4 (20)	

*Fisher's exact test; †Student t test; ‡ χ^2 test.

§All four tumours were located in the colon.

HPF, high power fields; R0, complete resection; R1, microscopic incomplete resection; R2, macroscopic incomplete resection.

Figure 2 Kaplan–Meier plots for survival of the 252 patients with gastrointestinal stromal tumours (GISTs) as a function of the risk of aggressive behaviour (A,B) and as a function of the KIT expression status (C,D). As the risk is higher, disease-free (A) and overall (B) survival rates become lower ($p < 0.001$ for both comparisons, log-rank tests). The cases of KIT-negative GIST had poorer disease-free (C) and overall (D) survival than those of KIT-positive GIST ($p = 0.153$ and $p = 0.042$, respectively, log-rank tests).

an epithelioid histology (fig 1); this was significantly higher than that of KIT-positive GISTs ($p < 0.001$). In terms of the risk of aggressive behaviour, KIT-negative GISTs more frequently belonged to the high-risk group than KIT-positive GISTs, but the difference was not significant. Also, KIT-negative GISTs more frequently manifested with metastatic disease at presentation and had a higher risk of dying of disease than KIT-positive GISTs. Because the cases were collected mainly from the pre-imatinib era, only 33 cases were treated with imatinib; 2 of these had KIT-negative GIST.

Table 4 Multivariate Cox proportional hazards model for the predictors of overall survival

Variables	Hazard ratio (95% CI)	p Value
KIT expression		0.030
KIT-negative	2.596 (1.097 to 6.147)	
KIT-positive	1.000	
Risk of aggressive behaviour		<0.001
Low and very low risk	0.075 (0.023 to 0.245)	
Intermediate risk	0.199 (0.078 to 0.508)	
High risk	1.000	
Imatinib treatment		0.017
No	3.531 (1.254 to 9.942)	
Yes	1.000	

Survival analyses

As expected, there was a significant correlation between risk of aggressive behaviour and the clinical outcome of patients. Kaplan–Meier curves for DFS and OS reflected the prognostic value of the histologically determined risk ($p < 0.001$ for both comparisons, log-rank tests; fig 2A,B), also demonstrating the accuracy of sampling of our patient group.

In the OS analysis of 78 patients with recurrent or metastatic disease, patients receiving imatinib treatment had a better survival rate than those not receiving it (median survival time, 24 vs 52 months; $p < 0.001$, log-rank test). Patients who had KIT-negative GIST had a worse prognosis than those having KIT-positive GIST ($p = 0.153$ for DFS and $p = 0.042$ for OS, log-rank for both comparisons; fig 2C,D). Patients with KIT-negative GISTs had a median duration of DFS of 31.5 (range 0–

Table 5 Immunohistochemical results for KIT-positive and KIT-negative gastrointestinal stromal tumours

	KIT-positive	KIT-negative	p Value*
CD34	195 (84.1)	7 (35.0)	<0.001
S-100 protein	24 (10.3)	1 (5.0)	0.443
Bcl-2	188 (95.9)	8 (40.0)	<0.001
PKCθ	216 (93.5)	15 (75.0)	<0.001

The number and percentage (in parentheses) of positive cases are reported for each marker.
 * χ^2 test.

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Table 6 Mutational status of KIT-positive and KIT-negative gastrointestinal stromal tumours

	KIT-positive (%)	KIT-negative (%)
<i>KIT</i> exon 9	3 (4.8)	0 (0)
<i>KIT</i> exon 11	31 (50.0)	0 (0)
<i>PDGFRA</i> exon 12	Not performed	0 (0)
<i>PDGFRA</i> exon 18	Not performed	6 (30.0)

165) months and a median duration of OS of 23 (range 1–165) months, as compared with 34 (range 0–179) and 53.5 (range 1–203) months, respectively, in patients with KIT-positive GIST. The 5-year OS rate was 68.7% (SD 10.7%) in patients with KIT-negative GIST, but 79.9% (SD 3.0%) in patients with KIT-positive GIST. The DFS rate at 5 years was 51.1% (SD 12.3%) in patients with KIT-negative GIST and 65.5% (SD 3.7%) in patients with KIT-positive GIST. Thus, negative KIT expression seems to be useful for defining a subgroup with unfavourable prognosis in GISTs. Also, multivariate Cox regression analysis for the prediction of OS revealed that negative KIT expression was an independent prognostic factor when the risk of aggressive behaviour and the status of imatinib treatment were adopted as covariates in the analysis (hazard ratio 2.596 (95% CI 1.097 to 6.147); table 4).

Immunohistochemical results of KIT-positive and KIT-negative GISTs

Table 5 presents immunohistochemical features of KIT-positive and KIT-negative GISTs. KIT immunostaining results of two representative cases of KIT-negative GIST are shown in fig 1 (B,D). All KIT-negative GISTs were completely negative for SMA and desmin. Moreover, KIT-negative GISTs showed lower expression rates for CD34, Bcl-2 and PKC θ than KIT-positive GISTs; these differences were statistically significant.

Mutational analyses of KIT-positive and KIT-negative GISTs

Mutational analyses on 62 KIT-positive cases revealed *KIT* exon 9 mutations in 4.8% and *KIT* exon 11 mutation in 50.0% (table 6). All exon 9 mutations were in-frame insertions of six nucleotides, which resulted in the duplication of alanine 501 and tyrosine 502. The most common type of exon 11 mutation (25 cases) was an in-frame deletion, which was sometimes accompanied by a point mutation. Mutational analysis of *PDGFRA* was not performed for KIT-positive GISTs. For KIT-negative GISTs, *PDGFRA* exon 18 mutations were found in six cases (30.0%; table 6); all mutations were missense mutations leading to the substitution of valine for aspartic acid 842 (D842V). The remaining 14 KIT-negative GISTs had no detectable *KIT* or *PDGFRA* mutation, although it should be

Figure 3 Frequency of protein kinase C theta (PKC θ) expression in gastrointestinal stromal tumours (GISTs) and non-GISTs by immunohistochemistry. PKC θ is expressed in 231 (93.9%) of 246 GISTs and in 14 (21.9%) of 64 non-GISTs.

noted that only exons 9 and 11 of *KIT* and exons 12 and 18 of *PDGFRA* were analysed in this study.

Table 7 describes the characteristics of the six KIT-negative GISTs harbouring a *PDGFRA* exon 18 mutation. Five of these six occurred in the stomach and showed an epithelioid histology. Of these five patients, one died of GIST. However, precise survival analysis was not possible because of the small number of patients in this group. All six cases were positive for PKC θ IHC. One case, a 53-year-old male patient, is illustrated in fig 1(A,B).

PKC θ expression status in GISTs and non-GISTs

In non-neoplastic regions, we detected positive cytoplasmic staining in interstitial cells of Cajal of the gastrointestinal tract and in the nerve plexus. In GISTs, PKC θ was expressed in 93.9% of available cases but in only 15 (75%) of 20 KIT-negative cases. PKC θ IHC showed diffuse cytoplasmic staining with weak, moderate or strong intensity in 21, 148 and 62 GISTs, respectively.

Interestingly, 14 (21.9%) of 64 non-GISTs also had expression of PKC θ (figs 3 and 4). The PKC θ -positive non-GISTs were as follows: 6/35 leiomyomas, 4/7 schwannomas, 2/7 leiomyosarcomas, and 2/13 desmoid tumours (fig 4C–F). However, the staining intensity of non-GISTs tended to be lower than that of GISTs; in PKC θ IHC of non-GIST cases, cytoplasmic staining with weak or moderate intensity was observed in 2 and 12 non-GISTs, respectively, and none showed strong staining intensity. Specifically, all four PKC θ -positive schwannomas showed multifocal staining with moderate intensity.

Table 7 Characteristics of the six KIT-negative gastrointestinal stromal tumours with a platelet-derived growth factor receptor α (*PDGFRA*) mutation

Age (y)/sex	Site	Size (cm)	Mitoses/50 HPF	Cell type	Survival	PKC θ IHC	<i>PDGFRA</i> mutation
55/M	Stomach	6.0	2	E	Alive	+	D842V
50/F	Omentum	28.0	25	E	Dead	+	D842V
48/M	Stomach	6.0	8	E	Alive	+	D842V
49/M	Stomach	4.5	2	S	Alive	+	D842V
62/M	Stomach	1.5	1	E	Alive	+	D842V
53/M	Stomach	3.0	1	E	Alive	+	D842V

HPF, high power fields; E, epithelioid; S, spindle; +, positive.

Figure 4 Representative immunohistochemical staining of protein kinase C theta (PKC θ). (A) PKC θ -positive gastrointestinal stromal tumour (GIST). (B) PKC θ -negative GIST. (C) PKC θ -negative leiomyoma. (D) PKC θ -positive schwannoma. (E) PKC θ -positive leiomyoma. (F) PKC θ -positive desmoid tumour. Original magnification, $\times 200$.

DISCUSSION

GIST is characterised by the expression of receptor tyrosine kinase KIT,² and KIT IHC positivity has been considered as a diagnostic feature of GIST.²⁻³ However, several reports have described a small subset of GISTs with little or no KIT expression.¹¹⁻¹² Recently, mutations of *KIT* and *PDGFRA* were identified in a subset of KIT-negative GISTs, which strongly supports the existence of KIT-negative GISTs. However, the diagnosis of KIT-negative GISTs without *KIT* or *PDGFRA* mutation is still problematic. In our study, the diagnostic criteria used for KIT-negative GISTs were a mesenchymal tumour consistent with GIST clinically and histologically and negativity for desmin and SMA by IHC. According to these criteria, 20 cases (7.9%) were categorised as KIT-negative GISTs; of these, 14 cases (70%) had no detectable *KIT* or *PDGFRA* mutation according to our mutational analysis.

The mutational rate of *KIT* and *PDGFRA* in KIT-negative GISTs according to our data is lower than previously reported.¹³⁻¹⁴ In our study, six KIT-negative GISTs harboured a mutation of *PDGFRA* exon 18; none showed a mutation of *PDGFRA* exon 12 and *KIT* exons 9 and 11. Medeiros *et al* identified a mutation of *KIT* exons 9 and 11 in one and three cases, respectively, and a mutation of *PDGFRA* exons 12, 14 and 18 in two, one and 15 cases, respectively, in a study of 25

KIT-negative GISTs; they also showed that none had a mutation of either exon 13 or 17 of *KIT*.¹³ Debiec-Rychter *et al* reported that three of seven KIT-negative GISTs harboured a mutation of *PDGFRA* exon 18 and none had any mutation of *KIT* exons 9, 11, 13, and 17 and *PDGFRA* exon 12.¹⁴ The relatively low mutational rate according to our results may be due to the small number of *KIT* and *PDGFRA* exons included in the mutational analysis, and the quality of paraffin-embedded tissues. Future mutational analyses of the entire coding region of *KIT* will be needed to elucidate the precise frequency of mutation and to investigate the possibility that mutations other than those of *KIT* and *PDGFRA* also underlie the pathogenesis of GIST. In addition, ploidy level analysis by fluorescence in situ hybridisation and karyotype analysis will be helpful in confirming the diagnosis of GIST for the cases with no *KIT* or *PDGFRA* mutation.¹⁴

KIT-negative GISTs are thought to differ from KIT-positive GISTs with respect to their clinicopathological, immunohistochemical and genetic features.¹³⁻¹⁵ Our large-scale study found that KIT-negative GISTs are more likely to originate in colon, omentum or peritoneum, have an epithelioid histology, and be classified as high risk. These clinicopathological findings were largely corroborated with previous reports on KIT-negative GISTs,¹³⁻¹⁵ but differ in that KIT-negative GISTs were more

likely to locate in colon besides omentum and peritoneum. However, this finding agrees with a report of Miettinen *et al*, documenting that only 76% of 25 GISTs in colon were positive for KIT.²³ In addition, we revealed that KIT-negative GISTs showed lower expression rates of CD34, Bcl-2, and PKC θ than KIT-positive GISTs, and 30% of KIT-negative GISTs harboured a *PDGFRA* mutation. Specifically, we characterised six cases of KIT-negative GISTs with a *PDGFRA* mutation. All harboured the classic D842V mutation of exon 18; most originated in stomach and had epithelioid cell histology, in agreement with previous reports.^{13–24}

Our study showed that patients with KIT-negative GISTs had a poorer OS rate than those with KIT-positive GISTs by log-rank test, and that negative KIT expression was an independent prognostic factor by multivariate Cox regression analysis for OS using the risk of aggressive behaviour and the status of imatinib treatment as covariates. These findings suggest that negative KIT protein expression may be associated with aggressiveness of tumour, although further study is required to elucidate this.

To the best of our knowledge, our report is the first to show that KIT expression status (positive vs negative) is an independent prognostic factor for OS of patients with GIST. Only a few reports stating survival differences according to KIT expression status could be found. Chirieac *et al* reported that there was a trend toward a superior 2-year progression-free survival in patients whose tumours demonstrated low KIT expression compared with those with strong KIT expression ($p = 0.012$, log-rank test) among 106 GIST patients who were treated with imatinib.²⁵ The results do not agree with our results, which may be due to the difference in study population (all were treated with imatinib in their report), or the difference in cut-off value used when classifying study cases according to KIT expression status. Although the number of KIT-negative GISTs is only 20 in this study, we anticipate that our findings will help to predict the clinical course of patients and define a subgroup with unfavourable prognosis in GIST. Results will be useful for selecting patients who are expected to benefit from imatinib therapy.

A subset of KIT-negative GISTs, such as GISTs with a *PDGFRA* mutation, was found to be responsive to imatinib.¹⁶ These findings emphasise that GIST in KIT-negative cases should not be missed. In addition, it was recently reported that a subset of GISTs without *KIT* or *PDGFRA* mutation was also responsive to imatinib.¹⁶ This observation suggests that mechanisms other than mutations of *KIT* or *PDGFRA* play important roles in the pathogenesis of GIST.

Recently, PKC θ IHC has been raised as a diagnostic marker of GIST, especially for KIT-negative GISTs.^{15–20} DNA microarray-based gene expression studies have revealed that the gene encoding PKC θ is overexpressed in GISTs,^{21–22} and IHC showed that PKC θ protein is highly expressed in GIST tissues.^{15–20} PKC is a serine/threonine kinase that controls cell proliferation, differentiation and motility.²⁶ Specifically, PKC θ is considered to be involved in T-cell activation,^{18–27} skeletal muscle signal transduction^{18–28} and neuronal differentiation.^{18–29} Some previous reports have suggested that PKC θ is not only sensitive but also specific as a diagnostic marker for GIST.^{15–20} PKC θ was expressed in 72–100% of GISTs on IHC in previous reports.^{15–20} The results for non-GIST tumours were variable; 47 non-GIST tumours,¹⁸ 2/10 leiomyosarcomas and 1/10 schwannomas,²⁰ 3/21 schwannomas and 0/4 leiomyosarcomas,¹⁵ and 4/10 melanomas and 4/15 schwannomas¹⁹ were positive for PKC θ IHC. The present study documented that PKC θ was

Take-home messages

- KIT-negative gastrointestinal stromal tumours (GISTs) are different from KIT-positive GISTs; KIT-negative GISTs were more likely to originate at the omentum or peritoneum, have an epithelioid histology, and be classified as high risk in this study.
- KIT-negative GISTs had a poorer overall survival rate than those with KIT-positive GISTs in Kaplan–Meier survival analysis. Negative KIT expression was an independent prognostic factor in multivariate Cox regression analysis for overall survival using the risk of aggressive behaviour and the status of imatinib treatment as covariates.
- While protein kinase C theta (PKC θ) immunohistochemistry is a sensitive diagnostic marker for GIST, its usefulness is limited because of the relatively low sensitivity in KIT-negative GISTs, and positivity in some tumours other than GIST.

expressed in 93.9% of 246 GISTs and also in 6/35 leiomyomas, 4/7 schwannomas and 2/7 leiomyosarcomas. The variety of the PKC θ results may be due to differences in IHC methods. Our study used autoclaving in Borg Decloaking Chamber (Biocare Medial), 1:15 of PKC θ antibody (BD Biosciences), and an avidin–biotin–peroxidase complex kit (Vectastatin Elite ABC Kit PK-6100); other studies used microwave or autoclaving, 1:50 or 1:100 of PKC θ antibody (BD Biosciences), and Dako EnVision+ System.^{18–20} In addition, the results of PKC θ IHC for KIT-negative GISTs were variable: 75% of the 20 cases exhibited PKC θ positivity in the present study; proportions were 72% of 18 in the study of Duensing *et al*,²⁰ all six of Motegi *et al*,¹⁵ and all four of Kim *et al*.¹⁹ The discrepancy of the results may be due to difference of selection criteria of KIT-negative GISTs besides differences of IHC methods.

According to our results of PKC θ IHC, we conclude that PKC θ is a sensitive marker, but its application as a diagnostic marker of GIST is limited because its expression was relatively low in KIT-negative GISTs and it was also expressed in some non-GISTs. Additional studies on larger populations are required to confirm these findings because of the low number of KIT-negative GISTs in the current study.

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REFERENCES

1. **Huizinga JD**, Thuneberg L, Kluppel M, *et al*. W/klt gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature* 1995;**373**:347–9.
2. **Sarlomo-Rikala M**, Kovatich AJ, Barusevicius A, *et al*. CD117: a sensitive marker for gastrointestinal stromal tumors that is more specific than CD34. *Mod Pathol* 1998;**11**:728–34.
3. **Fletcher CD**, Berman JJ, Corless C, *et al*. Diagnosis of gastrointestinal stromal tumors: a consensus approach. *Hum Pathol* 2002;**33**:459–65.
4. **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.
5. **Rubin BP**, Singer S, Tsao C, *et al*. KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res* 2001;**61**:8118–21.
6. **Corless CL**, Fletcher JA, Heinrich MC. Biology of gastrointestinal stromal tumors. *J Clin Oncol* 2004;**22**:3813–25.
7. **Hirota S**, Ohashi A, Nishida T, *et al*. Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. *Gastroenterology* 2003;**125**:660–7.
8. **Heinrich MC**, Corless CL, Duensing A, *et al*. *PDGFRA* activating mutations in gastrointestinal stromal tumors. *Science* 2003;**299**:708–10.
9. **Demetri GD**, von Mehren M, Blanke CD, *et al*. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med* 2002;**347**:472–80.

10. **van Oosterom AT**, Judson I, Verweij J, *et al*. Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. *Lancet* 2001;**358**:1421–3.
11. **Miettinen M**, Sobin LH, Sarlomo-Rikala M. Immunohistochemical spectrum of GISTs at different sites and their differential diagnosis with a reference to CD117 (KIT). *Mod Pathol* 2000;**13**:1134–42.
12. **Miettinen M**, Lasota J. Gastrointestinal stromal tumors—definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. *Virchows Arch* 2001;**438**:1–12.
13. **Medeiros F**, Corless CL, Duensing A, *et al*. KIT-negative gastrointestinal stromal tumors: proof of concept and therapeutic implications. *Am J Surg Pathol* 2004;**28**:889–94.
14. **Debiec-Rychter M**, Wasag B, Stul M, *et al*. Gastrointestinal stromal tumours (GISTs) negative for KIT (CD117 antigen) immunoreactivity. *J Pathol* 2004;**202**:430–8.
15. **Motegi A**, Sakurai S, Nakayama H, *et al*. PKC theta, a novel immunohistochemical marker for gastrointestinal stromal tumors (GIST), especially useful for identifying KIT-negative tumors. *Pathol Int* 2005;**55**:106–12.
16. **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.
17. **West RB**, Corless CL, Chen X, *et al*. The novel marker, DOG1, is expressed ubiquitously in gastrointestinal stromal tumors irrespective of KIT or PDGFRA mutation status. *Am J Pathol* 2004;**165**:107–13.
18. **Blay P**, Astudillo A, Buesa JM, *et al*. Protein kinase C theta is highly expressed in gastrointestinal stromal tumors but not in other mesenchymal neoplasias. *Clin Cancer Res* 2004;**10**:4089–95.
19. **Kim KM**, Kang DW, Moon WS, *et al*. PKCtheta expression in gastrointestinal stromal tumor. *Mod Pathol* 2006;**19**:1480–6.
20. **Duensing A**, Joseph NE, Medeiros F, *et al*. Protein kinase C theta (PKCtheta) expression and constitutive activation in gastrointestinal stromal tumors (GISTs). *Cancer Res* 2004;**64**:5127–31.
21. **Allander SV**, Nupponen NN, Ringner M, *et al*. Gastrointestinal stromal tumors with KIT mutations exhibit a remarkably homogeneous gene expression profile. *Cancer Res* 2001;**61**:8624–8.
22. **Nielsen TO**, West RB, Linn SC, *et al*. Molecular characterisation of soft tissue tumours: a gene expression study. *Lancet* 2002;**359**:1301–7.
23. **Miettinen M**, Sarlomo-Rikala M, Sobin LH, *et al*. Gastrointestinal stromal tumors and leiomyosarcomas in the colon: a clinicopathologic, immunohistochemical, and molecular genetic study of 44 cases. *Am J Surg Pathol* 2000;**24**:1339–52.
24. **Wardelmann E**, Hrychuk A, Merkelbach-Bruse S, *et al*. Association of platelet-derived growth factor receptor alpha mutations with gastric primary site and epithelioid or mixed cell morphology in gastrointestinal stromal tumors. *J Mol Diagn* 2004;**6**:197–204.
25. **Chirieac LR**, Trent JC, Steinert DM, *et al*. Correlation of immunophenotype with progression-free survival in patients with gastrointestinal stromal tumors treated with imatinib mesylate. *Cancer* 2006;**107**:2237–44.
26. **Nishizuka Y**. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science* 1992;**258**:607–14.
27. **Altman A**, Villalba M. Protein kinase C-theta (PKCtheta): it's all about location, location, location. *Immunol Rev* 2003;**192**:53–63.
28. **Hilgenberg L**, Miles K. Developmental regulation of a protein kinase C isoform localized in the neuromuscular junction. *J Cell Sci* 1995;**108**:51–61.
29. **Sparatore B**, Patrone M, Passalacqua M, *et al*. Neuronal differentiation of PC12 cells involves changes in protein kinase C-theta distribution and molecular properties. *Biochem Biophys Res Commun* 2000;**275**:149–53.

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