



Expression and prognostic role of molecular markers in 99 KIT-positive gastric stromal tumors in Taiwanese

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immunoreactivity ($P=0.026$), high PCNA LI ($P=0.015$) and male gender ($P=0.036$) were six predictors for early disease recurrence. Subsequent multivariate analysis revealed that mitotic counts, tumor size, and p53 immunoreactivity were three independent prognostic factors for both disease free and overall survival of patients. By combination of three independent prognostic factors for grouping, we found higher tumor recurrence rate ($P<0.001$) and shorter survival ($P<0.001$) existed in groups with increasing factors.

CONCLUSION: We first provide the prognostic value and linkage of three molecular markers in GISTs. The combination of three factors (p53, tumor size, and tumor mitosis) provides a more powerful prediction of prognosis than any single factor does.

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Keywords: Gastrointestinal stromal tumor; GIST; p53; PCNA; Ki-67; Prognosis

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Abstract

AIM: To elucidate the prognostic role and relationship of three molecular markers such as tumor suppressor gene p53, proliferating cell nuclear antigen (PCNA) and Ki-67 in gastric stromal tumor.

METHODS: A total of 108 surgically resected gastric smooth muscle tumor specimens were collected from January 1987 to December 1999. Immunohistochemical studies were performed on the paraffin sections of 99 of 108 CD117-positive tumors with antibodies of p53, PCNA, and Ki-67. Immunoreactivity of three molecular markers was recorded by labeling index (LI, %) and was analyzed for clinicopathologic and survival correlation.

RESULTS: Of the 99 cases, immunostaining revealed that 52 patients (52.5%) had p53, and 37 patients (37.3%) had Ki-67 immunoreactivity (defined as $>10\%$ of LI). All patients (100%) had PCNA immunoreactivity ranging from 12% to 93% of LI, divided into high or low by median. Statistics revealed that LI of three markers positively correlate to each other ($P<0.01$) and to microscopic tumor mitotic counts ($P<0.001$). By combination, patients with ≥ 2 markers (positive or high) in tumors had early tumor recurrence ($P<0.001$) and unfavorable outcome ($P<0.001$). Univariate analysis indicated that patients with tumor size >5 cm ($P=0.003$), tumor mitosis $>5/50$ HPF ($P<0.001$), p53 immunoreactivity ($P=0.001$), Ki-67

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are interstitial tumors arising in the wall of the gastrointestinal tract. The term, GIST was first introduced in the 1980s to include a group of non-lymphomatous, non-epithelial tumors of the gut^[1]. GISTs have strong morphological similarities in their pathological features, but have heterogeneous immunophenotype and biological behavior^[2]. In the past, spindle cell tumors of the gastrointestinal (GI) tract were usually classified as smooth muscle tumors (leiomyomas, or leiomyosarcomas). Over the past two decades, advances in pathology and molecular genetics have provided further evidences regarding the interstitial cells of Cajal as the progenitor cells for GIST^[3,4]. The use of c-kit receptor tyrosine kinase antibodies of KIT with a panel of other antibodies may distinguish true GIST from smooth muscle

Table 1 Sources of antibodies and ratio of dilutions used in this study

Antibody	Source	Dilution
CD117 monoclonal	Dako M7140 Clone104D2	1:50
CD 34 monoclonal	Dako M7161 CloneQBEnd10	1:200
Desmin monoclonal	Novocastra NCL-DES-DERII Clone DE-R-11	1:100
Smooth muscle actin monoclonal	Novocastra NCL-SMA Clone asm	1:50
S-100 polyclonal	Novocastra NCL-S100p	1:1 500
P53 monoclonal	Dako	1:200
Ki67 monoclonal	Dako MIB1	1:50
PCNA monoclonal	Dako M0879	1:50

tumors, neural tumors, and other spindle cell neoplasms^[5,7]. Thereafter, the gastric and intestinal smooth muscle tumors in the earlier data largely would be classified as GISTs by immunohistochemical methods^[5,7].

Concerning prognosis, many studies have established clinicopathologic correlations, yet the criteria claimed to predict the prognosis remains vague. Although the proliferating activity in terms of the mitotic count is generally accepted as a prognostic indicator^[1,8,10], other gross and histologic parameters have been reported to influence the course of disease, such as tumor size^[9-11], cellular density^[11,12], predominant cell type^[13,14], neural or muscular component^[13,15], necrosis^[10,12] and histological grades^[10,14,16]. It has been suggested that in addition to the use of KIT expression in the diagnosis of GIST, c-kit mutation may also correlate with the malignant potential of GIST^[17-19]. Molecular markers by immunohistochemical studies dealing with proliferating index like Ki-67^[20-23], proliferating cell nuclear antigen (PCNA)^[8,24,25], and tumor suppressor gene p53^[20,22,26], have been applied with encouraging results. However, the reports reflect a considerable controversy in considering the different conclusions of prognostic role in these molecular markers. Further, there were less linkage and elucidation of correlation between these genes in the previous literatures. Besides, there was a lack of documentation of KIT positive stromal tumor in the early reports that the conclusion might be misleading.

In our previous study of 81 gastric stromal tumors in the Taiwanese^[9], we have found that mitotic numbers and tumor sizes were two independent prognostic factors for disease free survival of patients. The conclusion was comparative to other studies. With the current investigation, we first elucidate the prognostic role of 99-CD117 (+) gastric stromal tumors by combination of three molecular markers such as tumor suppressor gene p53, PCNA and Ki-67. Their expression and correlation in GIST will be further verified in this original article.

MATERIALS AND METHODS

Subjects

A total of 108 surgically resected gastric smooth muscle tumor specimens were collected at Kaohsiung Chang-gung Memorial Hospital from January 1986 to December 1999. Patients with tumors which could not be completely

resected or patients with evident metastasis on diagnosis were excluded. All the tumors were obtained from curative resection and were diagnosed as leiomyoma or leiomyosarcoma by pathological studies. The diagnosis of malignant tumor was based on the index of mitosis as follows: benign < 5 and malignant $\geq 5/50$ high-power fields (HPF, single field area of 0.20 mm²). The closing date of follow-up was December 31, 2004. Patients who died due to post-surgical complications were excluded from the study. Data on age, gender, and follow up duration were collected. In each case, all slides were reviewed, and the following histological parameters were regarded and recorded by two pathologists: 1) cellularity (low, medium or high); 2) predominant cell type (spindle, epithelioid or mixed); 3) nuclear pleomorphism (mild, moderate or severe). Cell type was categorized as being predominantly spindle (>75% of the tumor), epithelioid (>75% of the tumor), or mixed if both the spindle and epithelioid components occupied >25% of the tumor. Nuclear pleomorphism, defined as variation in nuclear size and shape, was judged to be mild, moderate or severe, with the highest level of nuclear pleomorphism recorded in each tumor. The tumor locations were classified into three groups as antrum, body and fundi-cardiac areas.

Immunohistochemistry

Tissue specimens were maintained in formaldehyde-fixed, paraffin-embedded blocks. Sections stained with hematoxylin and eosin (HE) were also reviewed. The paraffin sections from the specimens were deparaffinized, blocked with 30 mL/L hydrogen peroxide for 10 min, and subjected to antigen retrieval with microwave in 0.01 mol/L citrate buffer for 15 min. The slides were then washed twice with PBS, incubated with primary antibodies of CD117, CD34, smooth muscle actin (SMA), S-100, desmin, Ki-67, p53 and PCNA for 30 min each, then examined with a peroxidase conjugate using polymer detection system (Zymed Cat. No. 87-89431) for 30 min. Specific details of the immunohistochemical condition used for each are shown in Table 1. The antibody staining was visualized with 3,3-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO, USA) in 0.1 mol/L Tris pH 7.2, containing 0.1 mL/L H₂O₂. The section slides were counterstained with Gill's hematoxylin, dehydrated, and mounted. Control tissues included mastocytoma of skin for positive control, and gastric carcinoma tissues and CD117 spared GIST for negative controls.

The markers (CD117, CD34, SMA, desmin, and S100) in the tumor cells on each slide were expressed as negative or as positive stain by estimating the number of positive tumor cells in the staining intensity. Negative was defined as no staining, or less than 10% of area with positive staining. Positive staining was defined as full or numerous staining. The labeling index (LI) of Ki-67, p53 and PCNA was calculated by two pathologists for each case. The percentage of Ki-67, p53, and PCNA-labeled tumor cells (from counting 1 000 cells) was calculated thrice for each tumor and an average of the three counts used for subsequent analyses. There was disagreement between proportion scores given by the two assessors in less than 5% of the cases for each immunostain. Any cases with

Table 2 The correlation between labeling index of molecular markers and pathologic parameters

	Tumor mitosis	Tumor size	P53	Ki67	PCNA
Tumor mitosis		CC=0.319 $P=0.001^1$	CC=0.488 $P<0.001^1$	CC=0.394 $P<0.001^1$	CC=0.657 $P<0.001^1$
Tumor size	CC=0.319 $P=0.001^1$		CC=0.285 $P=0.004^1$	CC=0.032 $P=0.751$	CC=0.206 $P=0.041^1$
P53	CC=0.488 $P<0.001^1$	CC=0.285 $P=0.004^1$		CC=0.434 $P<0.001^1$	CC=0.508 $P<0.001^1$
Ki67	CC=0.394 $P<0.001^1$	CC=0.032 $P=0.751$	CC=0.434 $P<0.001^1$		CC=0.295 $P=0.003^1$
PCNA	CC=0.657 $P<0.001^1$	CC=0.206 $P=0.041^1$	CC=0.508 $P<0.001^1$	CC=0.295 $P=0.003^1$	

CC: correlation coefficient. ¹Correlation is significant at the 0.05 level (two-tailed).

discrepant scores were reassessed to produce final scores for further analyses. The LI of Ki-67 and p53 more than 10% was defined as “positive” staining *vs* “negative” as less than 10%. Immunostaining of PCNA was recorded by percentage and was divided into two groups (by median) as high or low expression for further comparison.

Statistical analysis

The ages and tumor sizes were expressed as mean \pm SD. Comparisons between groups of independent samples were assessed by the Student's *t* test. Associations between categorical variables were assessed using χ^2 test. The correlation between continuous variables was determined by Spearman's rank correlation test. Survival rates were calculated by the Kaplan-Meier method and the difference in survival was compared with the log rank test. The influence of various clinicopathological features on survival was assessed by the Cox's proportional hazard model. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Of these 108 patients, nine had tumors lacking immunoreactivity for c-kit. These c-kit negative tumors were more likely to show positivity for smooth muscle markers or for S100 than the 99 c-kit positive tumors. Only one of the patients with c-kit negative tumors had tumor recurrence and died. The other eight patients had no disease recurrence at the end of follow-up. Finally, these nine patients were excluded for the study population of molecular markers. A total 99 cases of GISTs were included comprising 40 males and 59 females, with age ranging from 31 to 80 (mean: 58 \pm 9.7) years and tumor size ranging from 1 to 26 (mean 6.8 \pm 4.2) cm. There were 61 benign and 38 malignant tumors based on the microscopic mitotic activities. Tumors were located in the fundi-cardiac region in 52 cases, body area in 37 cases and antral area in 10 cases.

The median follow-up period was 60 (range 3-160) mo. At the end of follow-up, 24 of 99 patients (24.2%) had disease recurrence, and 23 patients died of tumors. Subsequent survival analyses revealed that patients with smaller tumor size, less mitotic activity, and female gender had lower recurrence rates ($P<0.05$) and longer survival

($P<0.05$) than patients with larger tumor size, more mitotic activity, and male gender (Figures 1A-1C; Tables 3 and 4). The 1-, 3-, and 5-year disease free survival rates were 100%, 93%, 93% (tumor <5 cm); 93%, 86%, 80% ($5 \leq$ tumor <10 cm); and 55%, 30%, 30% (tumor ≥ 10 cm), respectively. In addition, The 1-, 3-, and 5-year disease free survival rates were 98%, 89%, 89% (mitosis $<5/50$ HPF); 84%, 84%, 84% ($5 \leq$ mitosis $<10/50$ HPF); and 68%, 42%, 33% (mitosis $\geq 10/50$ HPF), respectively. Moreover, there was strong positive correlation between tumor sizes and tumor mitotic counts ($P=0.001$) (Table 2).

Subsequently, the results of immunostaining for p53, Ki-67 and PCNA were analyzed. The immunoreactivity of these markers was calculated by labeling index (LI, %). The results revealed that 52 patients (52.5%) had positive p53, and 37 patients (37.3%) had positive Ki-67 immunoreactivity (defined as more than 10%, Figure 2). All patients had positive PCNA immunoreactivity, ranging from 12% to 93%, with a median of 70% and mean of 66% (Figure 2). Statistical analyses revealed that the LI of three markers strongly correlate to each other. The *P* value was less than 0.001 for p53 *vs* PCNA; less than 0.001 for p53 *vs* Ki-67; and 0.003 for Ki-67 *vs* PCNA, respectively (Table 2). Furthermore, we also found that LI of all three markers strongly correlate to microscopic tumor mitotic counts (all $P<0.001$). But only LI of p53 ($P<0.001$) and PCNA ($P=0.04$) correlate to tumor sizes (Table 2). Besides that, p53 positivity also positively correlates to CD34 positivity ($P=0.001$), and high PCNA positively correlates to nuclear pleomorphism ($P=0.008$).

Univariate survival analysis revealed that patients with larger tumor size (≥ 5 cm, $P=0.003$), more tumor mitosis ($\geq 5/50$ HPF, $P<0.001$), positive p53 immunoreactivity ($P=0.001$), positive Ki-67 immunoreactivity ($P=0.026$), high PCNA LI (above median, $P=0.015$) and male gender ($P=0.036$) were six predictors for earlier disease recurrence (Figure 1, Table 3). In addition, these six factors also predict overall survival of patient, as tumor size ($P=0.004$), tumor mitosis ($P<0.001$), p53 immunoreactivity ($P=0.001$), Ki-67 immunoreactivity ($P=0.048$), PCNA LI ($P=0.025$), and male gender ($P=0.021$) (Table 4).

Further multivariate analysis by Cox's proportional hazard model revealed that only mitotic number, tumor size, and p53 immunoreactivity were independent prog-

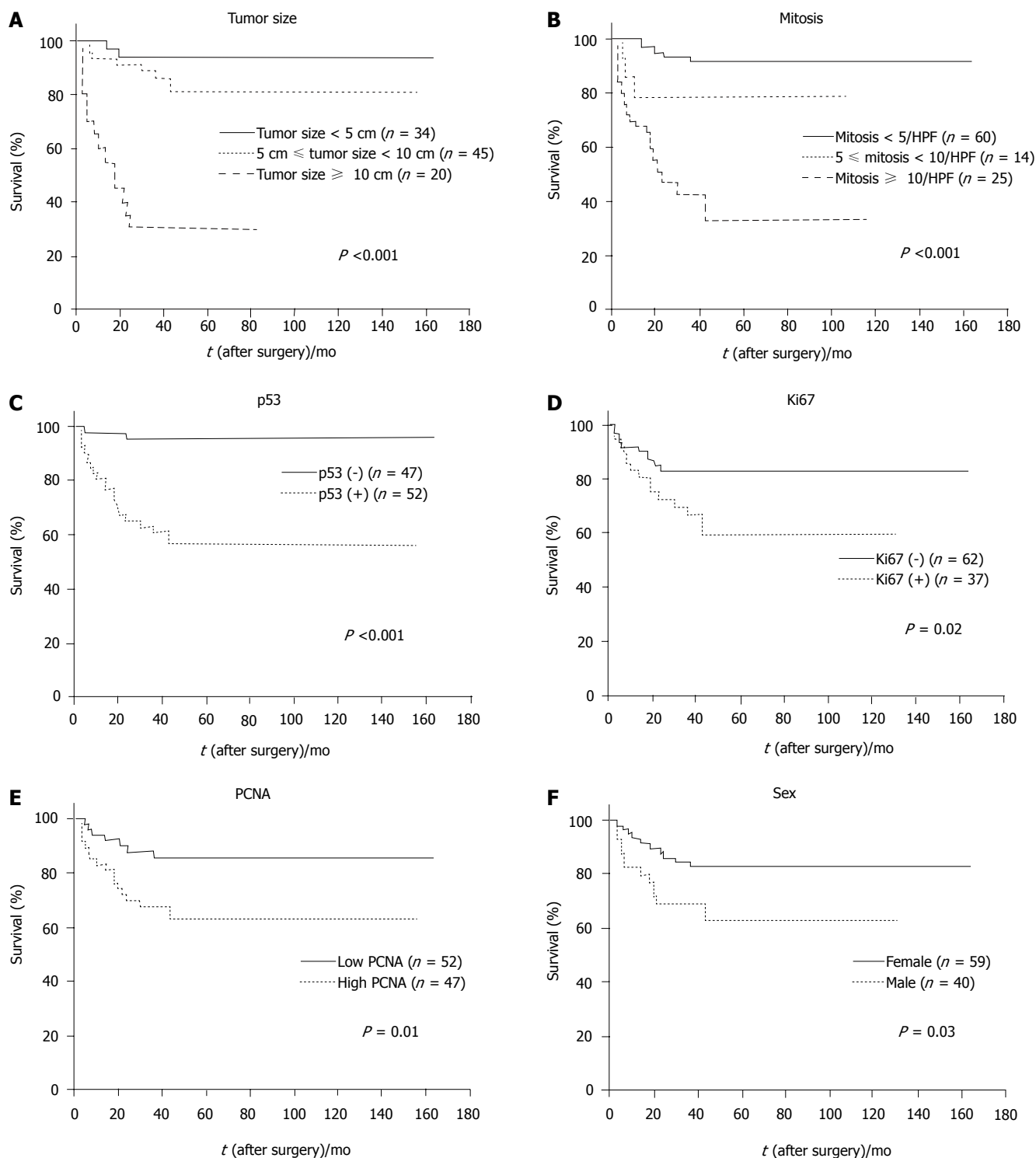


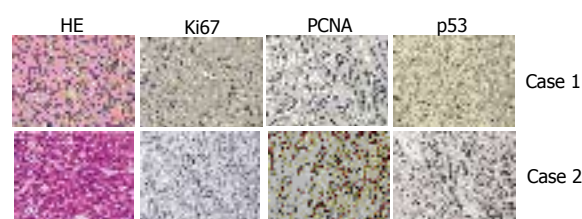
Figure 1 Kaplan-Meier analyses for patients survival (log rank test).

nostic factors for disease free and overall survival of GIST patients (Tables 3, 4). If we combine three molecular markers (p53 and Ki-67 positivity and high PCNA LI) for comparison, patients with more than two positive/high markers in their tumors had early tumor recurrence and unfavorable outcome (Figure 3A). Subsequently, we further combined three independent prognostic factors (p53 positivity; tumor size ≥ 5 cm; and tumor mitosis $\geq 5/50$ HPF) and divided the patients into four subgroups as 1) none of the event present; 2) one of the three events

present; 3) two of three events present and 4) all three events present. Statistical analysis revealed earlier tumor recurrence ($P < 0.001$) and shorter survival ($P < 0.001$) were found in the groups with increasing events. The 1-, 3-, 5-year recurrence rates were 0%, 0%, 0% (Group 1); 3.2%, 9.7%, 9.7% (Group 2); 4.5%, 15%, 15% (Group 3); and 42%, 67%, 77% (Group 4), respectively (Figure 3B). Further, 1-, 3-, 5-year overall survival rates were 100%, 100%, 100% (Group 1); 100%, 96%, 93% (Group 2); 95%, 90%, 83% (Group 3); and 62%, 37%, 22% (Group 4),

Table 3 Univariate and multivariate analyses for disease free survival rates of individual parameters of GIST patients

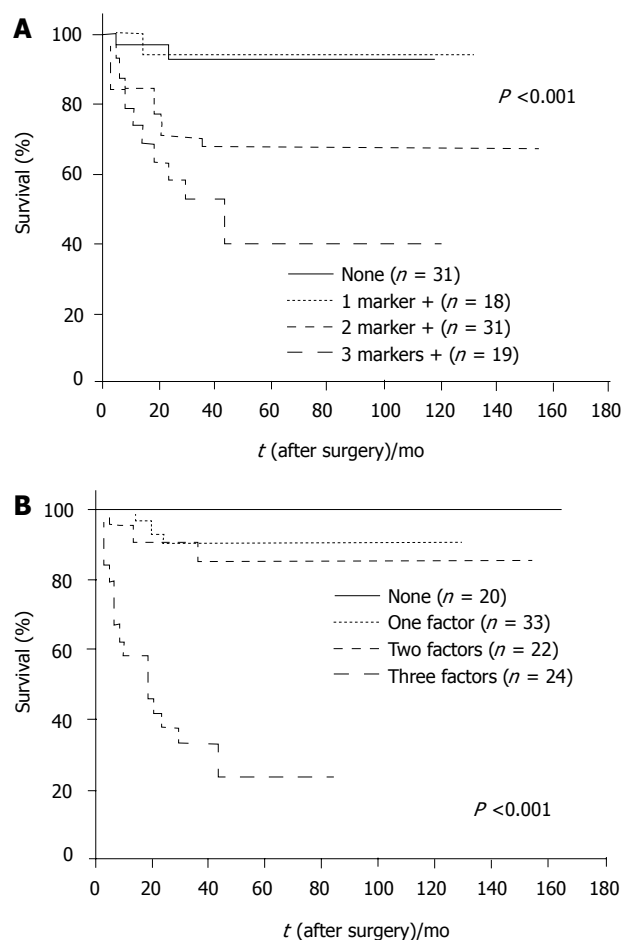
	Univariate			Multivariate		
	Risk	95%CI	P	Risk	95%CI	P
Age ≥ 60 / <60 yr	1.01	0.45-2.25	0.98	-	-	-
Gender M/F	2.38	1.05-5.36	0.036 ^a	-	-	0.1
Tumor size ≥ 5 / <5 cm	6.16	1.83-20.6	0.003 ^a	3.43	1.12-11.8	0.025 ^a
Mitoses ≥ 5 / <5 /50 HPF	6.30	2.49-15.9	<0.001 ^a	3.28	1.25-8.59	0.009 ^a
Tumor location fundic-cardiac/body-antrum	0.99	0.54-1.81	0.98	-	-	-
Hemoglobin ≥ 100 / <100	2.21	0.86-5.63	0.095	-	-	-
Cell type spindle/non-spindle	1.23	0.73-2.08	0.42	-	-	-
Cellularity low+intermediate/high	1.26	0.82-2.16	0.38	-	-	-
Pleomorphism +/-	0.82	0.36-1.89	0.65	-	-	-
P53 +/-	12.1	2.83-51.3	0.001 ^a	6.45	1.45-28.7	0.002 ^a
Ki67 +/-	2.52	1.12-5.68	0.026 ^a	-	-	0.41
PCNA above or below median	2.97	1.23-7.17	0.015 ^a	-	-	0.42
CD 34 +/-	3.07	0.41-22.7	0.27	-	-	-
SMA +/-	1.34	0.53-3.37	0.54	-	-	-
Desmin +/-	0.74	0.17-3.16	0.68	-	-	-
S-100 +/-	2.19	0.65-1.37	0.20	-	-	-

^a $P < 0.05$ vs others.**Figure 2** Immunohistochemical studies of p53, Ki67 and PCNA. Case 1 Negative p53, Ki-67 and low PCNA. Case 2 Positive p53, Ki-67 and high PCNA.

respectively. The combination of three factors provides a more powerful prediction of prognosis than any single factor does.

DISCUSSION

Several recent studies have suggested that tumor size and mitotic count, alone or in combination^[27,28], as well as Ki-67^[20,23-28] or PCNA^[8,24,25] proliferative index, and tumor suppressor gene p53^[20,22,26] are useful in predicting the clinical behavior of GIST. However, the varying conclusions drawn from previous studies are matched by varying deficiencies of these studies. First, relatively small case numbers were studied. Second, prognostic studies should not pool GISTs from different locations into a single heterogeneous group, but evaluate prognostic factors relating to each homogeneous tumor site. Moreover, previously published series^[11,28,29] indicated that distally located GISTs (small intestine, colon) seem to behave in more aggressive patterns than gastric tumors. Third, many studies were done before KIT detection was available. The distinction between GIST and pure neural or smooth muscle tumors is important as the latter may portend a better prognosis^[27,30]. Fourth, the reports reflect a considerable controversy in considering the different conclusions of prognostic role in these molecular markers.

**Figure 3** Combination of molecular markers and prognostic factors **A**: p53 and Ki-67 positivity and high PCNA LI for survival analysis (log rank test) prognostic factors **B**: p53 positivity; tumor size ≥ 5 cm; and tumor mitosis $\geq 5/50$ HPF.

In addition, there was also less linkage and elucidation of correlation between these genes in the previous literatures.

To overcome these deficiencies, we conducted a large

Table 4 Univariate and multivariate analyses for overall survival rates of individual parameters of GIST patients

	Univariate			Multivariate		
	Risk	95%CI	P	Risk	95%CI	P
Age ≥ 60 / <60 yr	0.90	0.39-2.06	0.81	-	-	-
Gender M/F	2.68	1.16-6.20	0.021 ^a	-	-	0.097
Tumor size ≥ 5 / <5 cm	19.01	2.56-141.1	0.004 ^a	10.17	1.34-76.9	0.001 ^a
Mitoses ≥ 5 / <5 /50 HPF	10.30	3.48-30.4	$<0.001^a$	5.22	1.70-15.9	0.001 ^a
Tumor location fundic-cardiac/body-antrum	1.07	0.58-1.96	0.81	-	-	-
Hemoglobin ≥ 100 / <100	1.98	0.78-5.05	0.14	-	-	-
Cell type spindle/non-spindle	1.29	0.76-2.18	0.34	-	-	-
Cellularity low+intermediate/high	1.21	0.64-2.17	0.35	-	-	-
Pleomorphism +/-	0.90	0.39-2.09	0.82	-	-	-
p53 +/-	11.29	2.64-48.2	0.001 ^a	4.84	1.08-21.6	0.012 ^a
Ki67 +/-	2.30	1.01-5.26	0.048 ^a	-	-	0.70
PCNA above or below median	2.75	1.13-6.70	0.025 ^a	-	-	0.74
CD 34 +/-	1.98	0.58-6.70	0.27	-	-	-
SMA +/-	1.38	0.54-3.52	0.48	-	-	-
Desmin +/-	0.76	0.18-3.27	0.72	-	-	-
S-100 +/-	2.28	0.67-7.70	0.18	-	-	-

cases review of 99 KIT-positive GISTs which were limited at the gastric area. By combination of these molecular markers and other clinicopathological factors, these results might shed a light to elucidate the clinical behavior of GIST. In the present study, we provided prognostic evidence of three molecular markers Ki-67, PCNA and p53 in GISTs. The expression of single gene closely correlates to each other. Further, each single gene had diagnostic value of malignant potential (correlation to tumor mitosis) of GIST. These findings suggested that high proliferative states as well as p53 positivity occur in a portion of GISTs and may constitute poor prognosis. The expression of tumor suppressor gene p53 correlated the proliferative states (PCNA, Ki-67, tumor mitosis), tumor sizes, and CD 34 positivity. Among these factors, p53 positivity, tumor mitosis, and tumor sizes were three independent prognostic factors. By combination of three independent prognostic factors, it provided the more powerful prediction for disease recurrence and survival of GIST patients than any individual factor did.

PCNA is a nuclear protein, which is closely related to the cell cycle regulation being an auxiliary molecule for DNA polymerase- δ ^[31]. It is also an auxiliary protein present during G₁-late phase and S phase. p53, a tumor suppressor gene, has an important function in DNA repair and in regulation of apoptosis. Mutations of p53 were described in malignant tumors and can be the cause of the alterations of this balance. The correlation between p53 and PCNA expression in other malignancies has been reported^[32,33]. Progression of neoplasia is often associated with the loss of cell cycle control. PCNA overexpression might predict more aggressive tumor behavior. In general, p53 positivity correlated with increased PCNA labeling index in neoplasia and represented advanced disease states and poor outcome. However, their correlation in GIST was not reported. In the present study, we first found that there was strong correlation between p53 and PCNA index in GIST, which was comparative with previous reports of other malignancies. It might be explained that the cyclin-

dependent kinase inhibitor p21/WAF1 is regulated by p53-dependent and p53-independent pathways. In addition, p21/WAF1 binds with PCNA and inhibits the action of PCNA^[34]. Overexpression of p53 on immunostaining (mostly p53 mutation) might disturb this pathway and trigger PCNA activity, thereby promoting cancer cells proliferation.

Of the three supplementary immunohistochemical markers tested, Ki-67 was most widely studied^[20-23,28] and it was also demonstrated in other malignancies^[35]. Ki-67 protein was considered to be more specific and reliable than PCNA as a marker of cell proliferation^[28]. However, two other studies reached significant conclusion by using PCNA instead of Ki-67 in GISTs^[24,36]. But they were all studied before KIT protein was available to detect GIST. In addition, whether Ki-67 or PCNA index is more practical and reliable than mitotic counting is still a matter of ongoing controversy. It has been suggested that there was less interobserver variation for Ki-67 index than for mitotic counting^[37]. But there was no comprehensive report dealing with the comparisons with PCNA. In the present study, the mitotic counting and immunostaining reading were performed by two pathologists to avoid interobserver bias. Based on the large case number of KIT-positive GISTs, our finding suggested that mitotic counting was still superior to Ki-67 or PCNA to predict prognosis in gastric GIST, even though they are closely related to each other. Although both Ki-67 and PCNA predict disease recurrence and overall survival of patients after the operation, they are not independent prognostic factors. Therefore, we are unable to demonstrate the priority of clinical application between Ki-67 and PCNA.

In studies of gastrointestinal smooth muscle tumors/GISTs, p53 positivity was a negative prognostic marker^[38,39]. However, p53 expression was prognostic in other studies^[20,40]. In our series, we found that p53 labeling index strongly related to three mitotic indices (Ki-67, PCNA, and tumor mitotic count) and represented a independent prognostic role in GIST patients. There

were several possible reasons for the discrepancy between these studies. First, different definition of positivity of p53 expression and different intensity of immunostaining read. Second, different dilutions and antigen retrieval methods were used. Third, whether disease related deaths were used as endpoints for survival analysis. To overcome this, we adopted a multifactorial method to evaluate the patients' prognosis. By combination of three molecular markers or three independent factors, the prediction of prognosis is more powerful than any single factor did. It might provide a further insight to deal with the clinical behavior of GIST in future. In conclusion, we demonstrated the first evidence of linkage between two proliferating markers and one tumor suppressor gene p53 in GISTs and the relationship between these markers and pathological mitotic counts and tumor size. By combination of these factors, it provided a more exact and powerful prediction of the prognosis of GIST patients after surgery.

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