SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 16: 3070-3082, 2018

# Role of PLK1 signaling pathway genes in gastrointestinal stromal tumors

JEN-SHI CHEN[1,2*], CHUN-NAN YEH[2,3*], CHI-TUNG CHENG[2-4], CHUEH-CHUAN YEN[5,6], YEN-YANG CHEN[7], SHIH-CHIANG HUANG[2,8], KUN-CHUN CHIANG[9], TA-SEN YEH[2,3], SAN-CHI CHEN[5,6], TA-CHUNG CHAO[5,6], MUH-HWA YANG[5,6] and YEE CHAO[5,6]

[1]Division of Hematology-Oncology, [2]GIST Team, [3]Department of Surgery, Linkou Chang Gung Memorial Hospital and Chang Gung University, Taoyuan 333; [4]Institute of Biomedical Informatics, [5]School of Medicine, National Yang-Ming University, Taipei 112; [6]Division of Medical Oncology, Center for Immuno-Oncology, Department of Oncology, Taipei Veterans General Hospital, Taipei 112; [7]Division of Hematology-Oncology, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung 833; [8]Department of Pathology, Linkou Chang Gung Memorial Hospital and Chang Gung University, Taoyuan 333; [9]Department of Surgery, Keelung Medical Center, Chang Gung Memorial Hospital and University, Keelung 204, Taiwan, R.O.C.

Received November 11, 2017; Accepted May 14, 2018

DOI: 10.3892/ol.2018.9003

**Abstract.** In previous studies by the authors, aurora kinase A (AURKA) was demonstrated as an independent poor prognostic marker for the recurrence of localized gastrointestinal stromal tumors (GISTs) and for the progression of advanced GISTs. In the present study, the prognostic effect of genes involved in cell cycle regulation in GISTs was further examined. Leading edge analysis in gene set enrichment analysis was used to identify the most common genes in the top 10 enriched gene sets of high-risk patients with GISTs in a Japanese study. The obtained gene list was uploaded to the Pathway Interaction Database to search for critical pathways. Selected genes within the pathway were subsequently verified through immunohistochemistry (IHC) in another cohort of patients. A total of 5 genes in 'PLK1 signaling events,' namely AURKA, polo-like kinase 1 (PLK1), cell division cycle 25C (CDC25C), budding uninhibited by benzimidazoles (BUB1), and targeting protein for Xklp2 (TPX2), were identified for subsequent study. Among the Japanese cohort, all 5 genes, except BUB1, were significant prognostic factors for poor recurrence-free survival (RFS). Among 141 patients enrolled for the IHC study, all 5 genes exhibited variable expression patterns. In the association study, only AURKA exhibited significant overexpression in non-gastric tumors. Although all 5 genes were considered as risk factors for poor RFS based on a univariate analysis, only the mitotic count and expression levels of CDC25C, BUB1, and TPX2 retained prognostic effects in the multivariate analysis. The PLK1 signaling pathway is crucial in the disease progression of GISTs. Genes within this pathway may serve as predictive markers for adjuvant therapy.

*Correspondence to:* Dr Chueh-Chuan Yen, Division of Medical Oncology, Center for Immuno-Oncology, Department of Oncology, Taipei Veterans General Hospital, 201 Sec 2, Shih-Pai Road, Taipei 112, Taiwan, R.O.C.
E-mail: ccyen@vghtpe.gov.tw

Dr Jen-Shi Chen, Division of Hematology-Oncology, Linkou Chang Gung Memorial Hospital and Chang Gung University, 5 Fu-Shun Street, Kwei-Shan, Taoyuan 333, Taiwan, R.O.C.
E-mail: js1101@cgmh.org.tw

*Contributed equally

*Key words:* aurora kinase A, budding uninhibited by benzimidazoles, cell division cycle 25C, gastrointestinal stromal tumor, polo-like kinase 1, targeting protein for Xklp2

## Introduction

Gastrointestinal stromal tumors (GISTs) are the most frequently observed intra-abdominal sarcomas and occur primarily from the gastrointestinal tract or less commonly from the mesentery and retroperitoneum (1-4). The majority of cases are characterized by an activated mutation of the receptor tyrosine kinase, KIT (5), or platelet-derived growth factor receptor α (6). Patients with advanced GISTs were successfully treated with imatinib mesylate, a potent tyrosine kinase inhibitor (TKI), with a median overall survival period of 5-6 years. However, acquired resistance mainly resulting from secondary mutation inevitably developed within 2-3 years after treatment (7,8). Salvage TKIs, including sunitinib maleate and regorafenib, yielded less satisfactory results (9,10). Therefore, novel biomarkers or treatment targets for TKI-refractory cases are required.

Genes that regulate cell cycle have a crucial role in predicting a prognosis for soft tissue sarcomas (11,12). By reanalyzing available expression profiling data on GISTs (13), our group has previously identified gene sets, including the cell cycle process or its regulation, that were strongly associated with the risk of recurrence. Aurora kinase A (AURKA) was identified as an independent poor prognostic marker for GIST

recurrence (14). A subsequent study by the authors confirmed that AURKA may also serve as an independent unfavorable marker for predicting disease progression or mortality from advanced GISTs and as a potential treatment target for GISTs (15). The aforementioned studies have implicated the crucial role of genes that regulate cell cycle in sarcomas and GISTs. In the present study, the prognostic effect of genes involved in cell cycle regulation in GISTs was examined.

## Patients and methods

*Bioinformatic analysis*. In a previous study by the authors (14), using gene set enrichment analysis (GSEA; downloaded from http://www.broadinstitute.org/gsea/index.jsp), 32 cases in Gene Expression Omnibus dataset GSE8167, previously reported by Yamaguchi *et al* (13), were classified into two risk groups with distinct recurrence-free survival (RFS) and expression profiles according to the modified criteria of Yen *et al* (14) from the Armed Forces Institute of Pathology (AFIP). Of the 715 Gene Ontology (GO) gene sets, which exhibited differential expression between two risk groups, 316 were upregulated in the high-risk phenotype. To identify significant genes and pathways in these gene sets, leading edge analysis (LEA) was used in GSEA to examine genes in the leading edge subsets of the top 10 enriched gene sets (14). It was hypothesized that genes that appear in multiple subsets are more likely to be of interest than those that appear in only one subset. The obtained gene list was uploaded to the Pathway Interaction Database (PID; https://wiki.nci.nih.gov/pages/viewpage.action?pageId=315491760) for analyzing the distribution of the molecules within predefined pathways. The query uses a hypergeometric distribution, which models the probability of observing *k* genes from a cluster of n genes (network frequency) by chance in a pathway or biological process category containing *m* genes from a total genome size of N genes (genome frequency), to compute the probability that each pathway in the database is hit by molecules in the list. It then returns a list of pathways ordered by P-value, which indicates the probability that the specific pathway is enriched by chance (16). The expression level of each individual probe was obtained using Z-score transformation. For genes encoded by more than one probe, average Z-score values were used for comparison. The differences among the risk groups were subsequently compared using the t-test.

*Tumor samples for immunohistochemistry study*. This study is a retrospective study. A total of 141 patients who received the diagnosis of GIST between 1989 and 2008 at Chang Gung Memorial Hospital (Taoyuan, Taiwan) were enrolled for immunohistochemistry (IHC) study. These cases were reported previously (14); they were patients with localized GIST who had received surgical excision only with no adjuvant imatinib therapy, had formalin-fixed paraffin-embedded tissues available for IHC study and were regularly followed up with appropriate radiological imaging evaluations at Chang Gung Memorial Hospital. The protocols for tumor sample collection and clinical record review were approved by the Institutional Review Board of Chang Gung Memorial Hospital (IRB no. 98-0352B), and all patients had provided informed consent for the use of their tissues and clinical data in research. All identifying information of individual patient was removed.

*IHC staining of cell cycle regulation genes in GIST*. A 4-$\mu$m section of each specimen was stained for selected proteins. Primary antibodies used in the present study were anti-Aurora (catalog no. ANB100-212; 1:1,500) and anti-polo-like kinase 1 (PLK1; catalog no. NBP1-02851; 1:100) antibodies from Novus Biologicals, (LLC, Littleton, CO, USA) as well as anti-cell division cycle 25C (CDC25C; catalog no. ab66235; 1:100), anti-budding uninhibited by benzimidazoles (BUB1; catalog no. ab4636; 1:100), and anti-targeting protein for Xklp2 (TPX2; catalog no. ab32795; 1:1,000) antibodies from Abcam, (Cambridge, UK). The antibodies were diluted as suggested and added to the slides, which were incubated overnight at 4˚C. The slides were then washed three times for 5 min each in a mixture of tris-buffered saline and Polysorbate 20 (the mixture is referred to as 'TBST' hereafter) prior to visualization using the LSAB2 system, Peroxidase (K0675; DAKO A/S). The control slides were incubated with the secondary antibody alone [also contained within the LSAB2 system, Peroxidase (K0675; DAKO A/S]. After 3 TBST washes for 5 min for each wash, the slides were mounted and blindly analyzed under microscopy by the authors. Immunostaining was scored.

For the assessment of IHC staining, the percentage of stained target cells was evaluated in 10 optical microscope fields per tissue section (magnification, x400) and the average staining percentage was calculated. For BUB1 and CDC25C, only nuclear staining was considered positive. Staining intensity was scored as 1 (mild), 2 (moderate), or 3 (intense). H-scores were calculated as the percentage of positive staining (0-100%) x the corresponding staining intensity (0-3) (17). The specimens with low and high H-scores were classified as having low and high IHC expression, respectively (cutoff value of H-scores: AURKA=60, TPX2=40, BUB1=50, CDC25C=50, and PLK1=60; Fig. 1).

*Statistical and survival analysis*. The associations between clinicopathological variables and IHC staining patterns of cell cycle regulation genes were analyzed using the $\chi^2$ test or Fisher's exact test for univariate analysis and using multinomial logistic regression for multivariate analysis. RFS was measured from the date of surgery to the date of tumor recurrence documentation. Survival analysis was estimated using the Kaplan-Meier method, and the log-rank test was conducted for survival curve comparison. The prognostic effect of clinicopathological factors and the IHC staining of genes that regulate cell cycle was determined by univariate and multivariate (stepwise forward conditional method) Cox regression analysis. In two-sided tests, $P<0.05$ was considered statistically significant. SPSS software (version 10.0; SPSS, Chicago, IL, USA) was used for all statistical analyses.

## Results

*Bioinformatic analysis identifies 5 critical genes and their expression levels are associated with risk groups and survival*. Among the top 10 GO gene sets (14), 15 genes were found in ≥9 of the sets (Table I). These 15 genes were uploaded to the

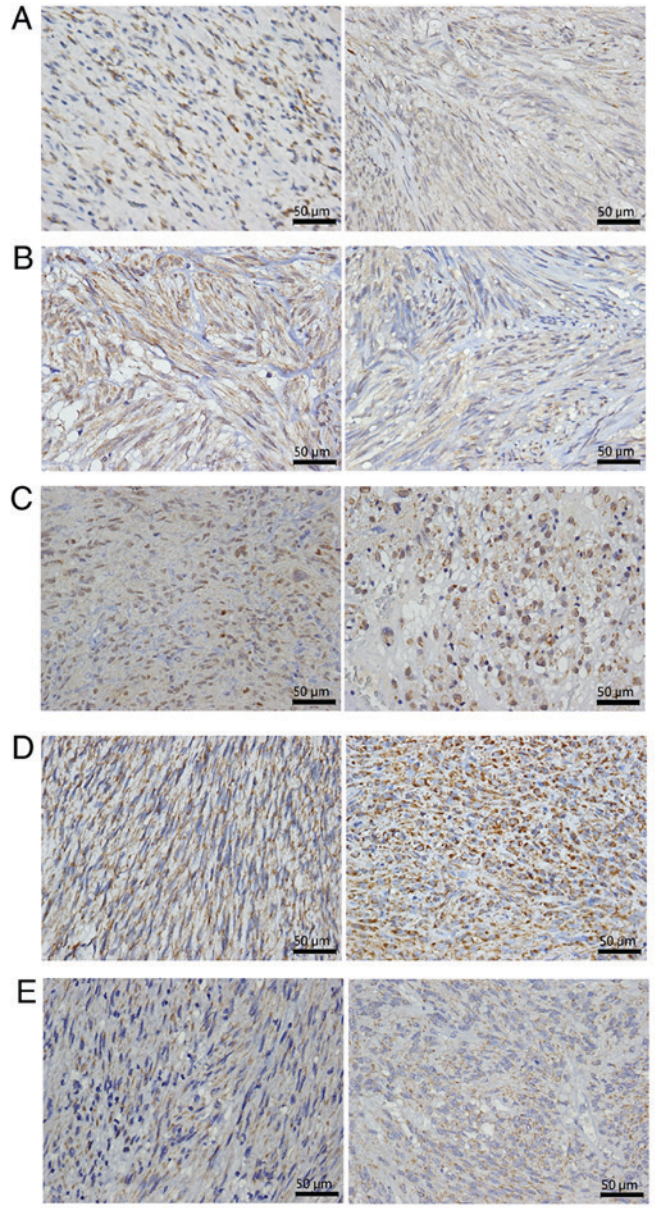


Figure 1. Examples of positive immunostaining of 5 genes (two figures are provided for each gene): (A) AURKA; (B) BUB1; (C) CDC25C; (D) PLK1; (E) TPX2. AURKA, aurora kinase A; BUB1, budding uninhibited by benzimidazoles 1 homolog; CDC25C, cell division cycle 25C; PLK1, polo like kinase 1.

PID, and 6 significant pathways were predicted. Among these pathways, 'PLK1 signaling events' was the most significant. A total of 5 genes-AURKA, PLK1, CDC25C, BUB1, and TPX2-were present in this pathway (Table II). These 5 genes were also identified in ≥8 GO gene sets that were associated with cell cycle regulation identified by gene set enrichment analysis (GESA) (Table I). Therefore, these 5 genes were selected for further study.

Differences in the expression levels of these 5 genes between the AFIP high-risk group and moderate- and low-risk groups were verified. As indicated in Fig. 2, the expression levels of the 5 genes, except for CDC25C, were significantly higher in the high-risk group compared with the moderate- and low-risk groups. Cox regression analysis revealed that patients with a high expression (Z score >0) of AURKA, PLK1, CDC25C or TPX2 had significantly lower RFS compared with those with

Table I. Identification of 15 genes by GSEA and the presence of these genes in the top 10 enriched gene sets.

| | KNTC1 | TTK | NEK2 | BUB1[a] | BIRC5 | AURKA[a] | MAD2L1 | ZWINT | ANLN | PLK1[a] | KIF2C | KIF11 | KIF15 | TPX2[a] | CDC25C[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mitosis | V | V | V | V | V | V | V | V | V | V | V | V | V | V | V |
| Cell cycle process | V | V | V | V | V | V | V | V | V | V | V | V | V | V | V |
| Mitotic cell cycle | V | V | V | V | V | V | V | V | V | V | V | V | V | V | V |
| M phase of mitotic cell cycle | V | V | V | V | V | V | V | V | V | V | V | V | V | V | V |
| Cell cycle phase | V | V | V | V | V | V | V | V | V | V | V | V | V | V | V |
| M phase | V | V | V | V | V | V | V | V | V | V | V | V | V | V | V |
| Microtubule cytoskeleton | V | V | V | V | V | V | | | V | V | V | V | V | V | |
| Cell cycle GO 0007049 | V | V | V | V | V | V | V | V | V | V | V | V | V | V | V |
| Regulation of mitosis | V | V | V | V | V | | V | V | V | | | | | | V |
| Non-membrane bound organelle | V | V | V | V | V | V | V | V | V | V | V | V | V | V | V |

'V' indicates the presence of gene in the GSEA gene sets. [a]Genes in the'PLK1 signaling events' identified by Pathway Interaction Database; GSEA, gene set enrichment analysis; ANLN, anillin actin binding protein; AURKA, aurora kinase A; BUB1, budding uninhibited by benzimidazoles 1 homolog; CDC25C, cell division cycle 25C; KIF11, kinesin family member 11; KIF15, kinesin family member 15; KNTC1, kinetochore associated 1; MAD2L1, mitotic arrest deficient 2 like 1; NEK2, NIMA related kinase 2; TPX2, targeting protein for Xklp2; TTK, phosphotyrosine picked threonine kinase; ZWINT, ZW10 interacting kinetochore protein.

Table II. Pathways identified by the Pathway Interaction Database.

| Pathway name | Biomolecules in the group | P-value |
|---|---|---|
| PLK1 signaling events | AURKA, BUB1, CDC25C, PLK1 and TPX2 | $2.16x10^{-9}$[a] |
| Aurora B signaling | AURKA, BIRC5, BUB1 and KIF2C | $2.05x10^{-7}$[a] |
| Aurora A signaling | AURKA, BIRC5 and TPX2 | $8.55x10^{-6}$[a] |
| FOXM1 transcription factor network | BIRC5, NEK2 and PLK1 | $2.16x10^{-5}$[a] |
| ATR signaling pathway | CDC25C and PLK1 | 0.00109[a] |
| p73 transcription factor network | BUB1 and PLK1 | 0.00474[a] |

[a]P<0.05 (hypergeometric distribution). AURKA, aurora kinase A; BUB1, budding uninhibited by benzimidazoles 1 homolog; CDC25C, cell division cycle 25C; TPX2, targeting protein for Xklp2; PLK1, polo like kinase 1.

Table III. Univariate analysis of the prognostic effect of expression level of the 5 genes on recurrence-free survival.

| Gene | Median survival, months (95% CI) | HR | Univariate analysis, 95% CI | P-value |
|---|---|---|---|---|
| AURKA | | | | |
| Low | Not reached | 1 | | 0.013[a] |
| High | 56.000 (21.635-90.365) | 7.242 | 1.527-34.345 | |
| PLK1 | | | | |
| Low | Not reached | 1 | | 0.006[a] |
| High | 53.000 (10.278-95.722) | 8.727 | 1.847-41.221 | |
| CDC25C | | | | |
| Low | Not reached | 1 | | 0.049[a] |
| High | 53.000 (6.515-99.485) | 3.685 | 1.008-13.475 | |
| BUB1 | | | | |
| Low | Not reached | 1 | | 0.082 |
| High | 53.000 (7.269-98.731) | 112.965 | 0.549-23248.895 | |
| TPX2 | | | | |
| Low | Not reached | 1 | | 0.001[a] |
| High | 28.000 (16.500-39.500) | 14.204 | 2.994-67.390 | |

[a]P<0.05, univariate Cox regression analysis. AURKA, aurora kinase A; BUB1, budding uninhibited by benzimidazoles 1 homolog; CDC25C, cell division cycle 25C; CI, confidence interval; HR, hazard ratio; TPX2, targeting protein for Xklp2.

low expression (Z score ≤0) of these 4 genes. The differences in survival between patients with different expression levels of BUB1 were of borderline significance (P=0.082) (Table III).

*Demographic and clinicopathological characteristics of patients enrolled for IHC study*. To validate the data from microarray analysis, the expression of the 5 genes (AURKA, PLK1, CDC25C, BUB1 and TPX2) were examined by using IHC in another group of patients with GIST. The demographic data and clinicopathological features of the 141 patients are listed in Table IV. In total, the group contained 74 men and 67 women with a mean age of 57.9 years (range, 22-89 years). The stomach constituted the most common tumor sites, and the mean tumor size was 7 cm. Under the AFIP criteria, ~35% of the patients were considered high risk.

*Association between the clinicopathological features and expression of the 5 genes in the IHC-validated patient cohort*. All 141 patients had IHC staining for AURAK and BUB1. Due to limited availability of tissue slides, IHC staining for TPX2, CDC25C and PLK1 were performed in 95, 121 and 140 patients, respectively. The association between the clinicopathological characteristics and expression pattern of the 5 genes in the IHC-validated patient cohort is illustrated in Tables V and VI. Higher AURKA expression was associated with non-gastric locations and a higher expression of BUB1, CDC25C and PLK1. Higher TPX2 expression was also associated with higher expression of BUB1, CDC25C and PLK1. Higher BUB1 IHC staining was associated with higher expression of 4 other mitotic check proteins similar to CDC25C and PLK1.

Multivariate analysis was performed in the 80 patients who had IHC for all 5 genes analyzed using multinomial logistic regression revealed that higher AURKA expression was independently associated with non-gastric locations and higher PLK1 expression. TPX2 staining was independently associated with higher PLK1 expression. BUB1 expression

Table IV. Clinicopathological characteristics of 141 patients with gastrointestinal stromal tumors.

| Clinicopathological features | Patients, n (%) |
|---|---|
| Mean age (years, mean ± SD) | 57.9±12.6 |
| Sex (M:F) | 74:67 |
| Location | |
| Gastric | 83 (58.9) |
| Non-gastric | 58 (41.1) |
| Tumor size (cm, mean ± SD) | 7.06±4.93 |
| Mitotic count, HPF | |
| <5/50 | 89 (63.1) |
| 5-10/50 | 16 (11.3) |
| >10/50 | 36 (25.5) |
| AFIP risk | |
| None | 10 (7.1) |
| Very low | 28 (19.9) |
| Low | 27 (19.1) |
| Moderate | 27 (19.1) |
| High | 49 (34.8) |
| AURKA immunostaining | |
| Low | 73 (51.8) |
| High | 68 (48.2) |
| TPX2 immunostaining | |
| Low | 64 (67.4) |
| High | 31 (32.6) |
| BUB1 nuclear immunostaining | |
| Low | 64 (45.4) |
| High | 77 (54.6) |
| CDC25C nuclear immunostaining | |
| Low | 55 (45.5) |
| High | 66 (54.5) |
| PLK1 immunostaining | |
| Low | 79 (56.4) |
| High | 61 (43.6) |

AFIP, Armed Forces Institute of Pathology; AURKA, aurora kinase A; BUB1, budding uninhibited by benzimidazoles 1 homolog; CDC25C, cell division cycle 25C; F, female; HPF, high-power fields; M, male; SD, standard deviation.

was independently associated with higher CDC25C and PLK1 expression. Higher CDC25C expression was independently associated with higher BUB1 expression. Higher PLK1 expression was independently associated with higher TPX2 and BUB1 expression (Table VII).

*Prognostic effect of clinicopathological factors and expression of 5 genes on RFS*. A total of 43 patients experienced recurrence, consisting of 11 locoregional relapses, 22 distant metastases and 10 multiple site recurrences. The univariate analysis indicated that the RFS was significantly affected by the tumor size, mitotic count, AFIP risk group classification, and expression level of the 5 aforementioned genes

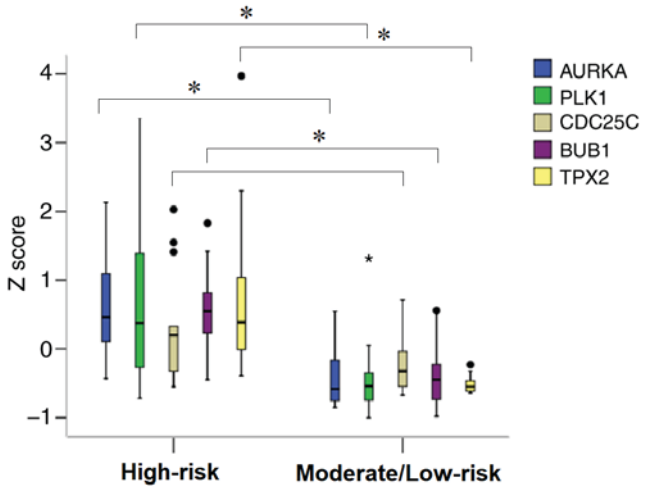


Figure 2. Comparison of expression level (indicated by Z scores) of 5 genes, AURKA, PLK1, CDC25C, BUB1 and TPX2, between the AFIP high- and moderate-/low-risk groups in 32 gastrointestinal stromal tumors (GIST) cases in the Japanese cohort using the t-test. With the exception of CDC25C, the expression level of the other 4 genes were significantly higher in the high-risk group compared with the moderate-/low-risk group. *P<0.05. AFIP, Armed Forces Institute of Pathology; AURKA, aurora kinase A; BUB1, budding uninhibited by benzimidazoles 1 homolog; CDC25C; cell division cycle 25C; PLK1, polo like kinase 1.

(Table VIII). However, only the mitotic count (P<0.001) and expression levels of TPX2 (P=0.008), BUB1 (P=0.023), and CDC25C (P=0.017) were identified as independent unfavorable prognostic factors for RFS through multivariate analysis (Table VIII). The Kaplan-Meier RFS curves for these 4 factors are shown in Fig. 3.

## Discussion

In the present analysis, LEA in GSEA was used to examine the genes in the leading edge subsets of the top 10 enriched gene sets and the resultant list of genes was uploaded to PID. A total of 5 genes-AURKA, PLK1, CDC25C, BUB1 and TPX2, were identified. In the patient cohort of dataset GSE8167, all but BUB1 were significant factors for poor RFS. In the IHC-validated patient cohort, only AURKA exhibited significant overexpression in non-gastric tumors when compared with gastric tumors (Table V). Although all 5 genes were considered risk factors for poor RFS based on the univariate analysis, only the expression levels of CDC25C, BUB1 and TPX2 together with mitotic count exhibited prognostic effects in the multivariate analysis.

Genes that are involved in the regulation of cell cycle have a crucial role in sarcomas. A prognostic gene expression signature-complexity index in sarcomas (CINSARC)-established by Chibon *et al* (12) and composed of 67 genes that are associated with chromosome integrity, mitotic control and genome complexity were able to predict metastasis outcomes. Similar findings were reported in studies of individual subtypes of sarcoma. In a study of uterine leiomyosarcomas (ULMSs) and their benign counterparts, Shan *et al* (18) showed that 26 of the 50 most overexpressed genes in ULMSs were involved in the regulation of mitosis and spindle function. On the other hand, our group (14) and Lagarde *et al* (11) have identified that genes

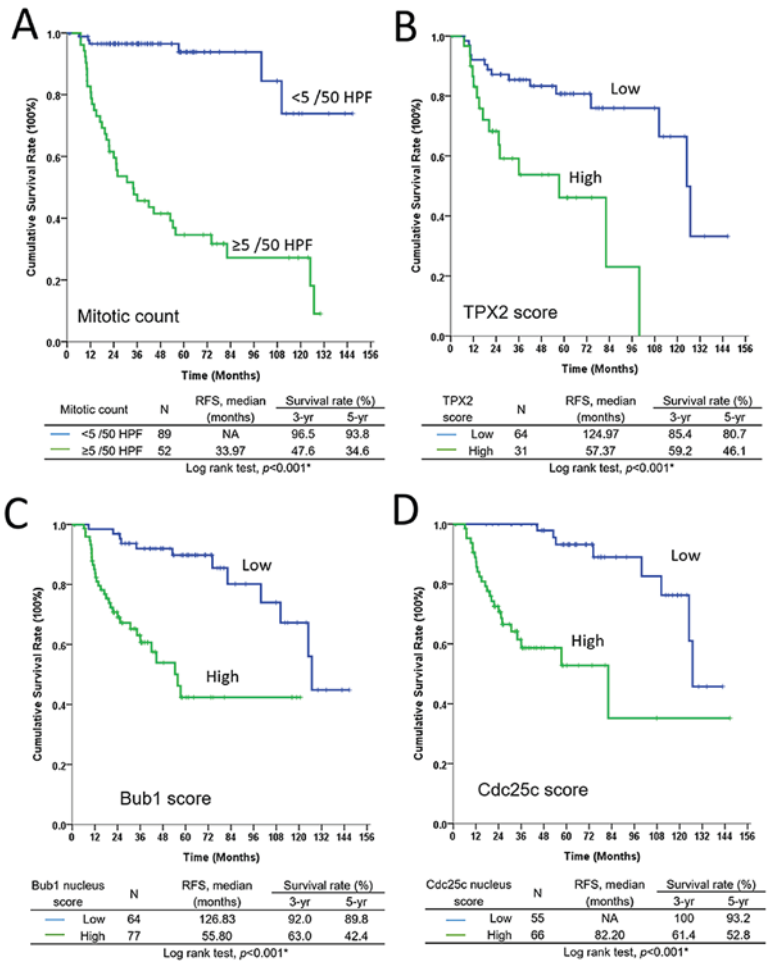


Figure 3. Kaplan-Meier plot of recurrence-free survival of patients with gastrointestinal stromal tumors according to: (A) Mitotic count (n=141); (B) TPX expression level (n=95); (C) BUB1 expression level (n=141); and (D) CDC25C expression level (n=121). The P-values for survival comparison, obtained by the log-rank test, were all <0.05. RFS, recurrence-free survival; TPX2, targeting protein for Xk; BUB1, budding uninhibited by benzimidazoles 1 homolog lp2; CDC25C; cell division cycle 25C.

that are involved in the progression or regulation of the cell cycle are strong prognostic factors of GIST recurrence. Most notably, all three studies have revealed that AURKA as one of the most crucial genes associated with ULMS and GIST recurrence (11,14,18).

In the present study, instead of random selection, LEA in GSEA was used in combination with PID to identify biologically relevant genes/pathways in GISTs. LEA in GSEA was performed to find genes that frequently appeared in the top 10 enriched gene sets. PID was used to search genes that are involved in pathways that are important in GISTs. 'PLK1 signaling events' was identified as a crucial pathway in GISTs, and 5 genes-AURKA, PLK1, CDC25, TPX2, and BUB1-in this pathway were examined.

AURKA and PLK1 are the key members of the Aurora kinase family and polo-like kinase family, respectively. Both proteins mainly function in the $G_2$-M phase of the cell cycle. In the late $G_2$ phase of the cell cycle, AURKA and PLK1 are recruited to the centrosome. PLK1 promotes the recruitment of AURKA that binds to centrosomin, whereas AURKA is responsible for the initial activation of PLK1 in $G_2$ (19). TPX2 is a protein that interacts with AURKA and can activate AURKA in the late $G_2$ and M phases (19). CDC25C is a phosphatase that is largely inactive in G2. However, mitotic entry is triggered by a steep increase in cyclin B-cyclin-dependent kinase 1 activity, which is promoted by PLK1-activated CDC25C (19). BUB1 is a critical component of the spindle assembly checkpoint, and the BUB1-PLK1 kinase complex promotes spindle checkpoint signaling through the phosphorylation of cell division cycle protein 20 (20). AURKA and PLK1 are involved in cytokinesis (19,20).

In the study that examined the associations among the expressions of the 5 genes, the expression levels of PLK1, BUB1, and CDC25C were observed to be highly associated. This finding is most likely because of the interaction between PLK1 and CDC25C during mitotic entry and the formation of BUB1-PLK1 complex during spindle assembly. In addition, the expression levels of PLK1 was closely associated with that of AURKA, indicating the interactive function of these proteins in the $G_2$-M phase (Table VII).

Schaefer *et al* (21) recently identified a tumor suppressor gene called MYC-associated factor X (MAX) in GISTs. MAX is localized on chromosome 14q, one of the most frequently deleted sites in GISTs (22). Inactivated mutations of MAX can be found in sporadic GISTs and patients with GISTs and neurofibromatosis type 1. These inactivated mutations of

Table V. Association of IHC intensity of five genes with clinicopathological characteristics of patients with gastrointestinal stromal tumors.

| | *AURKA* score, (%) | | *TPX2* score, (%) | | *BUB1* score, (%) | | *CDC25C* score, (%) | | *PLK1* score, (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Parameters | Low | High | Low | High | Low | High | Low | High | Low | High |
| Age | | | | | | | | | | |
| <60 | 38 (46.3) | 44 (53.7) | 34 (63.0) | 20 (37.0) | 37 (45.1) | 45 (54.9) | 31 (41.9) | 43 (58.1) | 48 (58.5) | 34 (41.5) |
| ≥60 | 35 (59.3) | 24 (40.7) | 30 (73.2) | 11 (26.8) | 27 (45.8) | 32 (54.2) | 24 (51.1) | 23 (48.9) | 31 (53.4) | 27 (46.6) |
| P-value | | 0.128 | | 0.293 | | 0.940 | | 0.323 | | 0.550 |
| Sex | | | | | | | | | | |
| Male | 35 (47.3) | 39 (52.7) | 35 (67.3) | 17 (32.7) | 33 (44.6) | 41 (55.4) | 23 (39.0) | 36 (61.0) | 40 (54.1) | 34 (45.9) |
| Female | 38 (56.7) | 29 (43.3) | 29 (67.4) | 14 (32.6) | 31 (46.3) | 36 (53.7) | 32 (51.6) | 30 (48.4) | 39 (59.1) | 27 (40.9) |
| P-value | | 0.264 | | 0.989 | | 0.842 | | 0.163 | | 0.549 |
| Location | | | | | | | | | | |
| Gastric | 53 (63.9) | 30 (36.1) | 36 (62.1) | 22 (37.9) | 36 (43.4) | 47 (56.6) | 33 (47.8) | 36 (52.2) | 43 (52.4) | 39 (47.6) |
| Non-gastric | 20 (34.5) | 38 (65.5) | 28 (75.7) | 9 (24.3) | 28 (48.3) | 30 (51.7) | 22 (42.3) | 30 (57.7) | 36 (62.1) | 22 (37.9) |
| P-value | | 0.001[a] | | 0.168 | | 0.565 | | 0.546 | | 0.258 |
| Tumor size, cm | | | | | | | | | | |
| <5 | 35 (59.3) | 24 (40.7) | 32 (76.2) | 10 (23.8) | 29 (49.2) | 30 (50.8) | 31 (56.4) | 24 (43.6) | 37 (63.8) | 21 (36.2) |
| 5-10 | 25 (48.1) | 27 (51.9) | 23 (63.9) | 13 (36.1) | 26 (50.0) | 26 (50.0) | 17 (38.6) | 27 (61.4) | 28 (53.8) | 24 (46.2) |
| >10 | 13 (43.3) | 17 (56.7) | 9 (52.9) | 8 (47.1) | 9 (30.0) | 21 (70.0) | 7 (31.8) | 15 (68.2) | 14 (46.7) | 16 (53.3) |
| P-value | | 0.288 | | 0.193 | | 0.161 | | 0.077 | | 0.275 |
| Mitotic count, HPF | | | | | | | | | | |
| <5/50 | 45 (50.6) | 44 (49.4) | 43 (71.7) | 17 (28.3) | 44 (49.4) | 45 (50.6) | 39 (49.4) | 40 (50.6) | 53 (60.2) | 35 (39.8) |
| ≥5/50 | 28 (53.8) | 24 (46.2) | 21 (60.0) | 14 (40.0) | 20 (38.5) | 32 (61.5) | 16 (38.1) | 26 (61.9) | 26 (50.0) | 26 (50.0) |
| P-value | | 0.706 | | 0.242 | | 0.207 | | 0.236 | | 0.238 |
| AFIP risk | | | | | | | | | | |
| Low | 38 (58.5) | 27 (41.5) | 31 (70.5) | 13 (29.5) | 32 (49.2) | 33 (50.8) | 32 (56.1) | 25 (43.9) | 37 (57.8) | 27 (42.2) |
| Moderate | 14 (51.9) | 13 (48.1) | 13 (72.2) | 5 (27.8) | 15 (55.6) | 12 (44.4) | 9 (36.0) | 16 (64.0) | 17 (63.0) | 10 (37.0) |
| High | 21 (42.9) | 28 (57.1) | 20 (60.6) | 13 (39.4) | 17 (34.7) | 32 (65.3) | 14 (35.9) | 25 (64.1) | 25 (51.0) | 24 (49.0) |
| P-value | | 0.256 | | 0.586 | | 0.152 | | 0.084 | | 0.576 |

[a]P<0.05 ($\chi^2$ or Fisher's exact test). AFIP, Armed Forces Institute of Pathology; HPF, high-power fields; AURKA, aurora kinase A; BUB1, budding uninhibited by benzimidazoles 1 homolog; CDC25C, cell division cycle 25C; PLK1, polo like kinase 1. All 141 patients have IHC staining of AURAK and BUB1. Due to limited availability of tissue slides, IHC staining for TPX2, CDC25C and PLK1 were performed in 95, 121 and 140 patients, respectively.

SPANDIDOS PUBLICATIONS
ONCOLOGY LETTERS 16: 3070-3082, 2018

Table VI. Association of IHC intensity between five genes.

| | *AURKA* score, (%) | | *TPX2* score, (%) | | *BUB1* score, (%) | | *CDC25C* score, (%) | | *PLK1* score, (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Low | High | Low | High | Low | High | Low | High | Low | High |
| *AURKA* score | | | | | | | | | | |
| Low | - | - | 29 (70.7) | 12 (29.3) | 41 (56.2) | 32 (43.8) | 35 (56.5) | 27 (43.5) | 50 (69.4) | 22 (30.6) |
| High | - | - | 35 (64.8) | 19 (35.2) | 23 (33.8) | 45 (66.2) | 20 (33.9) | 39 (66.1) | 29 (42.6) | 39 (57.4) |
| P-value | - | - | | 0.542 | | 0.008[a] | | 0.013[a] | | 0.001[a] |
| *TPX2* score | | | | | | | | | | |
| Low | - | - | - | - | 34 (53.1) | 30 (46.9) | 28 (51.9) | 26 (48.1) | 41 (64.1%) | 23 (35.9) |
| High | - | - | | | 7 (22.6) | 24 (77.4) | 6 (23.1) | 20 (76.9) | 6 (19.4) | 25 (80.6) |
| P-value | - | - | - | - | | 0.005[a] | | 0.015[a] | | <0.001[a] |
| *BUB1* score | | | | | | | | | | |
| Low | - | - | - | - | | - | 37 (66.1) | 19 (33.9) | 51 (79.7) | 13 (20.3) |
| High | - | - | - | - | - | - | 18 (27.7) | 47 (72.3) | 28 (36.8) | 48 (63.2) |
| P-value | - | - | - | - | - | - | | <0.001[a] | | <0.001[a] |
| *CDC25C* score | | | | | | | | | | |
| Low | - | - | - | - | - | - | - | | 41 (75.9) | 13 (24.1) |
| High | | - | - | - | - | - | - | - | 26 (39.4) | 40 (60.6) |
| P-value | | - | - | - | - | - | - | | | <0.001[a] |

[a]P<0.05 ($\chi^2$ or Fisher's exact test). AFIP, Armed Forces Institute of Pathology; HPF, high-power fields; AURKA, aurora kinase A; BUB1, budding uninhibited by benzimidazoles 1 homolog; CDC25C, cell division cycle 25C; PLK1, polo like kinase.

Table VII. Multivariate analysis of association of clinicopathological characteristics with IHC intensity of five genes in 80 GIST patients.

| | *AURKA* score | | *TPX2* score | | *BUB1* score | | *CDC25C* score | | *PLK1* score | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Odds ratio | P-value | Odds ratio | P-value | Odds ratio | P-value | Odds ratio | P-value | Odds ratio | P-value |
| Age (<60/≥60) | | NS | | NS | | NS | | NS | | NS |
| Gender (Male/Female) | | NS | | NS | | NS | | NS | | NS |
| Location (Gastric/Non-gastric) | 4.91 (2.02-11.94) | <0.001 | | NS | | NS | | NS | | NS |
| Tumor size (<5 cm/5-10 cm/>10 cm) | | NS | | NS | | NS | | NS | | NS |
| Mitotic count (<5/50 HPF/≥5/50 HPF) | | NS | | NS | | NS | | NS | | NS |
| AFIP risk (Low/Moderate/High) | | NS | | NS | | NS | | NS | | NS |
| AURKA score (Low/High) | | - | | NS | 1.73 (0.54-5.58) | 0.358 | 1.29 (0.45-3.73) | 0.633 | 1.67 (0.50-5.56) | 0.405 |
| TPX2 score (Low/High) | | NS | | - | 2.21 (0.59-8.26) | 0.24 | 1.56 (0.46-5.34) | 0.476 | 3.76 (1.05-13.51) | 0.042 |
| BUB1 score (Low/High) | 1.62 (0.63-4.16) | 0.317 | 2.36 (0.62-8.94) | 0.206 | | - | 3.38 (1.06-10.74) | 0.039 | 7.42 (2.27-24.29) | 0.001 |
| CDC25C score (Low/High) | 1.38 (0.57-3.34) | 0.481 | 1.58 (0.46-5.42) | 0.463 | 3.37 (1.05-10.76) | 0.041 | | - | 2.90 (0.88-9.58) | 0.081 |
| PLK1 score (Low/High) | 3.79 (1.41-10.18) | 0.008 | 3.92 (1.07-14.35) | 0.039 | 7.41 (2.26-24.34) | 0.001 | 2.84 (0.87-9.30) | 0.085 | | - |

Multinomial logistic regression was used for statistical analysis. AFIP, Armed Forces Institute of Pathology; AURKA, aurora kinase A; BUB1, budding uninhibited by benzimidazoles 1 homolog; CDC25C, cell division cycle 25C; HPF, high-power fields; PLK1, polo like kinase 1; NS, not significant.

Table VIII. Univariate and multivariate analyses of the prognostic effects of clinicopathological factors on recurrence-free survival.

| | Median survival | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|---|
| Factors | Months (95% CI) | HR | 95% CI | P-value | HR | 95% CI | P-value |
| Age | | | | | | | |
| <60 (n=82) | 124.97 (88.15-161.78) | 1 | | 0.580 | | | |
| ≥60 (n=59) | Not achieved | 1.189 | 0.644-2.194 | | | | |
| Sex | | | | | | | |
| Female (n=67) | Not achieved | 1 | | 0.104 | | | |
| Male (n=74) | 124.97 (52.27-197.66) | 1.672 | 0.900-3.104 | | | | |
| Location | | | | | | | |
| Gastric (n=83) | Not achieved | 1 | | 0.262 | | | |
| Non-gastric (n=58) | 110.27 (66.15-154.39) | 1.44 | 0.762-2.721 | | | | |
| Tumor size, cm | | | | | | | |
| <5 (n=59) | 124.97 (104.1-145.83) | 1 | | <0.001[a] | | | >0.05 |
| 5-10 (n=52) | 99.86 (59.64-140.10) | 4.899 | 1.646-14.583 | | | | |
| >10 (n=30) | 21.67 (15.17-28.17) | 18.436 | 6.324-53.740 | | | | |
| Mitotic count. HPF | | | | | | | |
| <5 /50 (n=89) | Not achieved | 1 | | <0.001[a] | 1 | | <0.001[a] |
| ≥5 /50 (n=52) | 33.97 (15.05-52.88) | 12.086 | 5.094-28.679 | | 9.207 | 2.958-28.661 | |
| AFIP risk | | | | | | | |
| Low (n=65) | Not achieved | 1 | | <0.001[a] | | | >0.05 |
| Moderate (n=27) | Not achieved | 12 | 1.401-102.793 | | | | |
| High (n=49) | 30.80 (17.57-44.04) | 56.914 | 7.791-415.752 | | | | |
| AURKA score | | | | | | | |
| Low (n=73) | Not achieved | 1 | | 0.040[a] | | | >0.05 |
| High (n=68) | 99.87 (53.50-146.23) | 1.904 | 1.031-3.517 | | | | |
| TPX2 score | | | | | | | |
| Low (n=64) | 124.97 (108.87-141.07) | 1 | | 0.001[a] | 1 | | 0.008[a] |
| High (n=31) | 57.37 (22.95-91.78) | 3.895 | 1.800-8.431 | | 4.016 | 1.440-11.196 | |
| BUB1 score | | | | | | | |
| Low (n=64) | 126.83 (122.04-131.63) | 1 | | <0.001[a] | 1 | | 0.023[a] |
| High (n=77) | 55.80 (39.99-71.61) | 5.038 | 2.419-10.494 | | 4.979 | 1.247-19.870 | |
| CDC25C score | | | | | | | |
| Low (n=55) | Not achieved | 1 | | <0.001[a] | 1 | | 0.017[a] |
| High (n=66) | 82.20 (22.76-141.64) | 6.484 | 2.786-15.091 | | 5.154 | 1.344-19.762 | |
| PLK1 score | | | | | | | |
| Low (n=79) | 126.83 (122.23-131.43) | 1 | | 0.001[a] | 1 | | >0.05 |
| High (n=61) | 54.40 (35.35-73.45) | 3.132 | 1.615-6.071 | | | | |

Univariate and multivariate Cox regression analyses were performed. [a]P<0.05. AFIP, Armed Forces Institute of Pathology; HR, hazard ratio; CI, confidence interval; AURKA, aurora kinase A; BUB1, budding uninhibited by benzimidazoles 1 homolog; CDC25C, cell division cycle 25C; HPF, high-power fields; PLK1, polo like kinase 1.

MAX were also detected in micro-GISTs, indicating that it is an early event (21).

MAX is a binding partner of MYC and has been reported as a tumor suppressor in hereditary pheochromocytomas and small-cell lung cancer (23,24). Previous studies have demonstrated that MAX may impair MYC function by impairing the ability of MYC to bind to DNA (25,26). MYC is a crucial regulator of cell cycle progression. Schaefer *et al* (21) demonstrated that the inactivation of MAX resulted in the silencing of the p16 gene, possibly via MYC activation (27). In addition, MYC induces cell proliferation that is generally associated with increases in the activity of CDK2, CDK4 and CDK6 to regulate $G_1$-S phase progression (28). Therefore, it was hypothesized that the inactivation of the MAX tumor suppressor occurs early

in GIST progression and leads to p16 inactivation and increased proliferation by enhancing $G_1$-S phase progression (21). Further cell cycle dysregulation in high-risk GISTs is most likely due to the overexpression of genes in the PLK1 signaling pathway, which may be a result of mutations of other tumor suppressor genes (29-31) with subsequent increased progression of the $G_2$-M phase and transition to high-grade cancer (22).

In addition to the expression levels of the aforementioned 5 genes, the clinicopathological factors that associated with recurrence included tumor size, mitotic count and AFIP risk group classification. This finding is reasonable as all these factors are considered risk factors for recurrence. In the multivariate analysis, only mitotic count and the expression levels of TPX2, BUB1 and CDC25C were identified as independent factors for poor RFS. TPX2, BUB1 and CDC25C have been previously reported to have prognostic effects on many types of cancer but not on sarcomas or GISTs (32-38). However, unexpectedly the multivariate analysis in the present study did not reveal AURKA and PLK1 to have statistically significant effects on RFS. In a previous study by the authors, AURKA expression was associated with non-gastric tumor (14). In the present study, after including the expression of 4 other genes in the multivariate analysis, AURKA expression remained independently associated with non-gastric locations. This result indicated that AURKA might be responsible for a distinct and more rapidly deteriorating clinical course of non-gastric GISTs.

Other molecules involved in cell cycle regulation that may be associated with the progression of GISTs have been previously reported. For example, in an European Organization for Research and Treatment of Cancer (EORTC) study, impaired p53, p16, BCL2 and CHK2 expression was commonly detected in advanced GISTs (29). Alteration of genes involved in $G_2$-M phase of cell cycle, including cyclin A, cyclin B1 and cdc2, were identified to be markers for predicting the aggressive behavior of GISTs in a Japanese study (39). These studies further supported the important roles of cell cycle regulators in GISTs.

There are limitations of statistical analysis in the present study. Due to limited availability of tissue slides, only AURKA and BUB1 IHC staining were done in all 141 patients. IHC staining for TPX2, CDC25C and PLK1 were done in 95, 121 and 140 patients, respectively. This definitely jeopardized the final analysis of this study. A total of 43 patients experienced recurrence. This relatively low number of recurrence may limit the statistical power of the study. Hopefully, there may be a larger cohort of patients through collaboration of multiple hospitals for further validation in the future. Another issue is that the possibility of multicollinearity (40) when using Cox regression model for multivariate analysis cannot be ruled out, since there is a high number of interactions between all the genes in cell cycle regulation. Nonetheless, CDC25C and BUB1, the two genes identified as independent prognostic factors, were also the only two genes that could be found in the GESA GO gene set 'regulation of mitosis' (Table I), indicating their critical roles in the disease. This result demonstrated that the present study was still able to identify important genes through Cox regression analysis.

In conclusion, through bioinformatics analysis and IHC validation, 5 genes-AURKA, PLK1, CDC25C, BUB1 and TPX2-in the PLK1 signaling pathway were identified as risk factors for poor prognosis of GIST. AURKA was significantly overexpressed in non-gastric GISTs. All 5 genes were considered as risk factors for poor RFS based on the univariate analysis. The mitotic count and expression levels of CDC25C, BUB1 and TPX2 retained prognostic effects in the multivariate analysis. The results of the present study indicated that the PLK1 signaling pathway might be crucial in the disease progression of GISTs. Furthermore, genes in this pathway may serve as predictive markers for adjuvant therapy.

**Acknowledgements**

Not applicable.

**Funding**

The present study was supported by grants from the Department of Health in Taiwan (Center of Excellence for Cancer Research, Taipei Veterans General Hospital, grant nos. DOH99-TD-C-111-007 and DOH100-TD-C-111-007 and the National Research Program for Biopharmaceuticals, grant no. DOH100-TD-PB-111-TM026), from the National Science Council (grant nos. NSC 100-2314-B-075-081 and NSC 101-2314-B-075-029), from the Ministry of Science and Technology, (grant nos. MOST 103-2314-B-075-066, MOST 105-2314-B-075-059 and MOST 106-2314-B-075-065), from the Taipei Veterans General Hospital (grant nos. V97ER2-010, V98ER2-004, V102E8-003, V103E8-001, V101C-133, V102C-034, V103C-188, V104C-099, V104E16-001-MY3-1, V104E16-001-MY3-2, V104D16-001-MY3-3, V105C-094 and V106C-160) and from the Yen Tjing Ling Medical Foundation (grant nos. CI-100-19, CI-103-6 and CI-105-4) in Taiwan to Dr CC Yen, as well as by grants from the Bayer and the Chang Gung Medical Research Program XMRPG3D0411, XPRPG3D0412, XPRPG3D0413 and XPRPG3D0414 to Dr CN Yeh. Dr CC Yen and Dr CN Yeh were also partially supported by grants from the Taiwan Clinical Oncology Research Foundation as well as Chong Hin Loon Memorial Cancer and Biotherapy Research Center of National Yang-Ming University, and the Taiwanese Society of Molecular Medicine, respectively.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors' contributions**

JC, CNY, CC and CCY were responsible for study design. SH was the pathologist and responsible for the pathology slide review and scoring. YC, KC and TY were members of the GISTs team from Chang Gung Memorial Hospital and responsible for clinical data collection. SC, TC, MY and YC were responsible for data analysis. JC and YC were also responsible for the completion of final manuscript.

**Ethics approval and consent to participate**

The protocols for tumor sample collection and clinical record review were approved by the Institutional Review Board of

Chang Gung Memorial Hospital (IRB no. 98-0352B), and all patients had provided informed consent for the use of their tissues and clinical data in research. All identifying information of individual patient was removed.

### Patient consent for publication

Consent for publication of data and any associated images was received from patients.

### Conflict of interest

The authors declare that they have no competing interest.

### References


1. Tran T, Davila JA and El-Serag HB: The epidemiology of malignant gastrointestinal stromal tumors: An analysis of 1,458 cases from 1992 to 2000. Am J Gastroenterol 100: 162-168, 2005.
2. Miettinen M, Monihan JM, Sarlomo-Rikala M, Kovatich AJ, Carr NJ, Emory TS and Sobin LH: Gastrointestinal stromal tumors/smooth muscle tumors (GISTs) primary in the omentum and mesentery: Clinicopathologic and immunohistochemical study of 26 cases. Am J Surg Pathol 23: 1109-1118, 1999.
3. Miettinen M and Lasota J: Gastrointestinal stromal tumors: Review on morphology, molecular pathology, prognosis, and differential diagnosis. Arch Pathol Lab Med 130: 1466-1478, 2006.
4. Reith JD, Goldblum JR, Lyles RH and Weiss SW: Extragastrointestinal (soft tissue) stromal tumors: An analysis of 48 cases with emphasis on histologic predictors of outcome. Mod Pathol 13: 577-585, 2000.
5. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, *et al*: Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 279: 577-580, 1998.
6. Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, Singer S, Griffith DJ, Haley A, Town A, *et al*: PDGFRA activating mutations in gastrointestinal stromal tumors. Science 299: 708-710, 2003.
7. Blanke CD, Demetri GD, von Mehren M, Heinrich MC, Eisenberg B, Fletcher JA, Corless CL, Fletcher CD, Roberts PJ, Heinz D, *et al*: Long-term results from a randomized phase II trial of standard-versus higher-dose imatinib mesylate for patients with unresectable or metastatic gastrointestinal stromal tumors expressing KIT. J Clin Oncol 26: 620-625, 2008.
8. Yeh CN, Chen YY, Tseng JH, Chen JS, Chen TW, Tsai CY, Cheng CT, Jan YY and Chen MF: Imatinib mesylate for patients with recurrent or metastatic gastrointestinal stromal tumors expressing KIT: A decade experience from Taiwan. Transl Oncol 4: 328-335, 2011.
9. Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, McArthur G, Judson IR, Heinrich MC, Morgan JA, *et al*: Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: A randomised controlled trial. Lancet 368: 1329-1338, 2006.
10. Demetri GD, Reichardt P, Kang YK, Blay JY, Rutkowski P, Gelderblom H, Hohenberger P, Leahy M, von Mehren M, Joensuu H, *et al*: Efficacy and safety of regorafenib for advanced gastrointestinal stromal tumours after failure of imatinib and sunitinib (GRID): An international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet 381: 295-302, 2013.
11. Lagarde P, Pérot G, Kauffmann A, Brulard C, Dapremont V, Hostein I, Neuville A, Wozniak A, Sciot R, Schöffski P, *et al*: Mitotic checkpoints and chromosome instability are strong predictors of clinical outcome in gastrointestinal stromal tumors. Clin Cancer Res 18: 826-838, 2012.
12. Chibon F, Lagarde P, Salas S, Pérot G, Brouste V, Tirode F, Lucchesi C, de Reynies A, Kauffmann A, Bui B, *et al*: Validated prediction of clinical outcome in sarcomas and multiple types of cancer on the basis of a gene expression signature related to genome complexity. Nat Med 16: 781-787, 2010.
13. Yamaguchi U, Nakayama R, Honda K, Ichikawa H, Hasegawa T, Shitashige M, Ono M, Shoji A, Sakuma T, Kuwabara H, *et al*: Distinct gene expression-defined classes of gastrointestinal stromal tumor. J Clin Oncol 26: 4100-4108, 2008.
14. Yen CC, Yeh CN, Cheng CT, Jung SM, Huang SC, Chang TW, Jan YY, Tzeng CH, Chao TC, Chen YY, *et al*: Integrating bioinformatics and clinicopathological research of gastrointestinal stromal tumors: Identification of aurora kinase A as a poor risk marker. Ann Surg Oncol 19: 3491-3499, 2012.
15. Yeh CN, Yen CC, Chen YY, Cheng CT, Huang SC, Chang TW, Yao FY, Lin YC, Wen YS, Chiang KC, *et al*: Identification of aurora kinase A as an unfavorable prognostic factor and potential treatment target for metastatic gastrointestinal stromal tumors. Oncotarget 5: 4071-4086, 2014.
16. Schaefer CF, Anthony K, Krupa S, Buchoff J, Day M, Hannay T and Buetow KH: PID: The pathway interaction database. Nucleic Acids Res 37: D674-D679, 2009.
17. Hirsch FR, Varella-Garcia M, Bunn PA Jr, Di Maria MV, Veve R, Bremmes RM, Barón AE, Zeng C and Franklin WA: Epidermal growth factor receptor in non-small-cell lung carcinomas: Correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol 21: 3798-3807, 2003.
18. Shan W, Akinfenwa PY, Savannah KB, Kolomeyevskaya N, Laucirica R, Thomas DG, Odunsi K, Creighton CJ, Lev DC and Anderson ML: A small-molecule inhibitor targeting the mitotic spindle checkpoint impairs the growth of uterine leiomyosarcoma. Clin Cancer Res 18: 3352-3365, 2012.
19. Lens SM, Voest EE and Medema RH: Shared and separate functions of polo-like kinases and aurora kinases in cancer. Nat Rev Cancer 10: 825-841, 2010.
20. Jia L, Li B and Yu H: The Bub1-Plk1 kinase complex promotes spindle checkpoint signalling through Cdc20 phosphorylation. Nat Commun 7: 10818, 2016.
21. Schaefer IM, Wang Y, Liang CW, Bahri N, Quattrone A, Doyle L, Mariño-Enríquez A, Lauria A, Zhu M, Debiec-Rychter M, *et al*: MAX inactivation is an early event in GIST development that regulates p16 and cell proliferation. Nat Commun 8: 14674, 2017.
22. Schaefer IM, Mariño-Enríquez A and Fletcher JA: What is new in gastrointestinal stromal tumor? Adv Anat Pathol 24: 259-267, 2017.
23. Romero OA, Torres-Diz M, Pros E, Savola S, Gomez A, Moran S, Saez C, Iwakawa R, Villanueva A, Montuenga LM, *et al*: MAX inactivation in small cell lung cancer disrupts MYC-SWI/SNF programs and is synthetic lethal with BRG1. Cancer Discov 4: 292-303, 2014.
24. Comino-Méndez I, Gracia-Aznárez FJ, Schiavi F, Landa I, Leandro-Garcia LJ, Letón R, Honrado E, Ramos-Medina R, Caronia D, Pita G, *et al*: Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma. Nat Genet 43: 663-667, 2011.
25. Maltais L, Montagne M, Bédard M, Tremblay C, Soucek L and Lavigne P: Biophysical characterization of the b-HLH-LZ of ΔMax, an alternatively spliced isoform of Max found in tumor cells: Towards the validation of a tumor suppressor role for the Max homodimers. PLoS One 12: e0174413, 2017.
26. Comino-Méndez I, Leandro-García LJ, Montoya G, Inglada-Pérez L, de Cubas AA, Currás-Freixes M, Tysoe C, Izatt L, Letón R, Gómez-Graña Á, *et al*: Functional and in silico assessment of MAX variants of unknown significance. J Mol Med (Berl) 93: 1247-1255, 2015.
27. Tabor V, Bocci M, Alikhani N, Kuiper R and Larsson LG: MYC synergizes with activated BRAFV600E in mouse lung tumor development by suppressing senescence. Cancer Res 74: 4222-4229, 2014.
28. Malumbres M: Physiological relevance of cell cycle kinases. Physiol Rev 91: 973-1007, 2011.
29. Romeo S, Debiec-Rychter M, Van Glabbeke M, Van Paassen H, Comite P, Van Eijk R, Oosting J, Verweij J, Terrier P, Schneider U, *et al*: Cell cycle/apoptosis molecule expression correlates with imatinib response in patients with advanced gastrointestinal stromal tumors. Clin Cancer Res 15: 4191-4198, 2009.
30. King SI, Purdie CA, Bray SE, Quinlan PR, Jordan LB, Thompson AM and Meek DW: Immunohistochemical detection of Polo-like kinase-1 (PLK1) in primary breast cancer is associated with TP53 mutation and poor clinical outcom. Breast Cancer Res 14: R40, 2012.
31. Li Z, Sun Y, Chen X, Squires J, Nowroozizadeh B, Liang C and Huang J: p53 mutation directs AURKA overexpression via miR-25 and FBXW7 in prostatic small cell neuroendocrine carcinoma. Mol Cancer Res 13: 584-591, 2015.
32. Hsu PK, Chen HY, Yeh YC, Yen CC, Wu YC, Hsu CP, Hsu WH and Chou TY: TPX2 expression is associated with cell proliferation and patient outcome in esophageal squamous cell carcinoma. J Gastroenterol 49: 1231-1240, 2014.

33. Glaser ZA, Love HD, Guo S, Gellert L, Chang SS, Herrell SD, Barocas DA, Penson DF, Cookson MS and Clark PE: TPX2 as a prognostic indicator and potential therapeutic target in clear cell renal cell carcinoma. Urol Oncol 35: 286-293, 2017.
34. Tomii C, Inokuchi M, Takagi Y, Ishikawa T, Otsuki S, Uetake H, Kojima K and Kawano T: TPX2 expression is associated with poor survival in gastric cancer. World J Surg Oncol 15: 14, 2017.
35. Wang Z, Katsaros D, Shen Y, Fu Y, Canuto EM, Benedetto C, Lu L, Chu WM, Risch HA and Yu H: Biological and clinical significance of MAD2L1 and BUB1, genes frequently appearing in expression signatures for breast cancer prognosis. PLoS One 10: e0136246, 2015.
36. Sun Q, Zhao H, Zhang C, Hu T, Wu J, Lin X, Luo D, Wang C, Meng L, Xi L, *et al*: Gene co-expression network reveals shared modules predictive of stage and grade in serous ovarian cancers. Oncotarget 8: 42983-42996, 2017.
37. Li L, Xu DB, Zhao XL and Hao TY: Combination analysis of Bub1 and Mad2 expression in endometrial cancer: Act as a prognostic factor in endometrial cancer. Arch Gynecol Obstet 288: 155-165, 2013.
38. Wang Z, Trope CG, Florenes VA, Suo Z, Nesland JM and Holm R: Overexpression of CDC25B, CDC25C and phospho-CDC25C (Ser216) in vulvar squamous cell carcinomas are associated with malignant features and aggressive cancer phenotypes. BMC Cancer 10: 233, 2010.
39. Nakamura N, Yamamoto H, Yao T, Oda Y, Nishiyama K, Imamura M, Yamada T, Nawata H and Tsuneyoshi M: Prognostic significance of expressions of cell-cycle regulatory proteins in gastrointestinal stromal tumor and the relevance of the risk grade. Hum Pathol 36: 828-837, 2005.
40. Alin A: Multicollinearity. Wiley Interdiscip Rev Comput Stat 2: 370-374, 2010.