Positive cyclin T expression as a favorable prognostic factor in treating gastric gastrointestinal stromal tumors

LIEN-FU LIN[1], JONG-SHIAW JIN[2], JUI-CHANG CHEN[3], CHIA-CHI HUANG[4], JENG-HORNG SHEU[3], WENLUNG CHEN[3], TANG-YI TSAO[2] and CHIH-WEI HSU[3,5]

[1]Division of Gastroenterology, Department of Internal Medicine; [2]Department of Pathology, Tungs' Taichung MetroHarbor Hospital, Taichung 435; [3]Department of Applied Chemistry, National Chia-Yi University, Chia-Yi 600; [4]Center of Nano Bio-Detection, National Chung Cheng University Chia-Yi, Chia-Yi 621; [5]Division of General Surgery, Department of Surgery, Tungs' Taichung MetroHarbor Hospital, Taichung 435, Taiwan, R.O.C.

Received May 20, 2015; Accepted October 26, 2015

DOI: 10.3892/mco.2016.835

Abstract. Positive transcriptional elongation factor b (P-TEFb) contains the catalytic subunit cyclin-dependent kinase 9 (Cdk9) and the regulatory subunit cyclin T. Cyclin T1 and Cdk9 are the key factors of the PTEFb pathways and are overexpressed in the human head and neck carcinoma cell line. However, there have been limited studies regarding the role of cyclin T1 and Cdk9 in gastric gastrointestinal stromal tumors (GISTs). The aim of the present study was to assess the association between cyclin T1 and Cdk9 and their clinical significance in gastric GISTs. A total of 30 gastric GIST patients who underwent either laparoscopic or laparotomic partial gastrectomy were enrolled in the study. The surgical tissue slides were stained with Cdk9 and cyclin T1 antibodies, and the immunohistochemistry scores and disease-free survival (DFS) were analyzed. Ten patients were cyclin T1-positive, and 20 were negative. All 11 patients with recurrent tumors or distant metastases were cyclin T1-negative patients. Old age, large tumor size, a high Ki67 IHC staining score, high mitotic count and negative cyclin T1 staining revealed a worse clinical outcome in univariate analysis. By contrast, the Cdk9 score was not associated with clinical parameters. The Kaplan-Meier survival curve illustrated that the DFS rate of the patients with negative cyclin T1 staining was significantly lower than that of the patients with positive cyclin T1 staining. Positive expression of cyclin T1 was a good prognostic factor in patients with gastric GISTs.

Correspondence to: Dr Chih-Wei Hsu, Division of General Surgery, Department of Surgery, Tungs' Taichung MetroHarbor Hospital, 699, Sec 8, Taiwan Boulevard, Wuqi, Taichung 435, Taiwan
E-mail: b8201076@ms42.hinet.net

Key words: gastric gastrointestinal stromal tumor, cyclin T1, cyclin-dependent kinase 9, disease-free survival

Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract, and mesenchymal tumors are thought to constitute ~1% of primary gastrointestinal cancers (1,2). Between 50-70% of GISTs arise from the stomach, which is the most common site of origin (1,3). The National Institutes of Health (NIH) 2002 consensus stratified GIST risk according to tumor size and mitotic rate (3). It has been proposed that these stratification guidelines should be widely used to predict recurrent risk factors, and modifications have also been proposed (4); however, certain factors regarding the criteria remain to be elucidated, such as the impact of tumor location, which may lead to an overestimation of the risk of large gastric GISTs (5,6). Another risk stratification schema that incorporates the impact of tumor location has been supported by three large retrospective studies conducted by the Armed Forced Institute of Pathology (7-9). However, some questions remain regarding the rare clinical settings that lack proposed parameters of risk assessment, as the data was too insufficient to lead to a convincing definition (5,6). Therefore, finding a reliable immunohistochemical predictor would be helpful for clinical practice.

Positive transcriptional elongation factor b (P-TEFb) contains the catalytic subunit cyclin-dependent kinase 9 (Cdk9) and the regulatory subunit cyclin T. Cyclin T contains subunits T1, T2a and T2b. Cdk9 is complexed with T1, T2a and T2b in ~80, 10 and 10% of cases, respectively (10-12). The expression pattern of cyclin T2a almost completely overlapped the pattern described for cyclin T1 (12). The expression of cyclin T1 increased the Cdk9 kinase activity and the phosphorylation of RNA polymerase II (RNAPII). The hypophosphorylation of the carboxyl-terminal repeat domain of RNAPII promotes and starts the elongation phase of transcription (13). This enables RNAPII to escape promoter-proximal pausing in order to engage the factors for pre-mRNA processing (14).

P-TEFb has been proficiently investigated in cardiac hypertrophy and human immunodeficiency virus (HIV) infection. The transcriptional activation for HIV-1 requires Tat interaction with human cyclin T1 and involves the formation of a complex with P-TEFb to increase the amount of RNAPII (15).

In addition, the Cdk inhibitor, flavopiridol, has been reported to have a mitigating effect on RNAPII levels (16). By contrast, hypertrophic signals may activate Cdk9 and consequently cause phosphorylation of RNAPII. This effect not only increases RNA synthesis but also enlarges myocyte size and may result in cardiac hypertrophy (13,14). In previous years, certain studies have reported that P-TEFb and transcriptional elongation exhibited key roles in protecting normal and cancerous cells from apoptosis (17). Cdk9 inhibitors have been thought to be potential therapeutic agents for chronic lymphocytic leukemia (18) and lung adenocarcinoma (19). Cyclin T1 and Cdk9 are also overexpressed in the human head and neck carcinoma cell lines (10).

The expression of cyclins A, B, D and E appear to be associated with high-grade disease but not with the clinical outcome (20-22). In addition, cyclin H-positive patients have a poor prognosis when they have high-risk GIST and when they exhibit metastasis or recurrent disease (23). However, there are few effective clinical predictors for gastric GIST. The aim of the present study was to assess the association of cyclin T1 and Cdk9, and the clinical parameters of gastric GISTs.

Materials and methods

Tissues and patients. The study included 30 gastric GIST patients who underwent either laparoscopic or laparotomic partial gastrectomy between 2008 and 2011 by the same surgical team at Tungs' Taichung MetroHarbor Hospital (Taiwan, China). Fifteen of the patients who were at a high risk of recurrence according to the NIH consensus received postoperative adjuvant chemotherapy with imatinib, and 1 patient received sunitinib. The medical charts, pathological reports, and surgical notes of all the study participants were retrospectively reviewed. The pathological diagnoses were reviewed by at least two experienced pathologists.

Ethics statement. The study was granted approval from the Institutional Review Board of the Tungs' Taichung MetroHarbor Hospital. All the patients included in the study received a full explanation of the procedures involved and provided written informed consent prior to collection of the specimens and clinical information.

Immunohistochemical staining. Using the Bond-Max autostainer (Leica Microsystems, Buffalo Grove, IL, USA), the slides were stained with Cdk9 monoclonal antibody and cyclin T1 polyclonal antibody. The details of these immunomarkers, including methods of pretreatment for antigen retrieval, are provided in Table I. Briefly, formalin-fixed and paraffin-embedded tissue specimens were placed in Tris-buffered saline and Tween 20, rehydrated through serial dilutions of alcohol, and washed in phosphate-buffered saline (pH 7.2), which was the buffer that was used for all subsequent washes, according to the manufacturer's recommended protocol. The coated slides were stained with the previously mentioned antibodies, and the immunostaining procedure was performed on the fully-automated Bond-Max system using the onboard heat-induced antigen retrieval and a Leica Refine Polymer Detection System (Leica Microsystems). Diaminobenzidine was used as the chromogen (Leica Microsystems) in all these immunostainings. Negative controls were obtained by excluding the primary antibody. Appropriate positive controls were used throughout the study. These slides were mounted with gum for microscopic examination, and the images were captured by the Olympus BX51 microscopic/DP71 Digital Camera System (Olympus, Ina, Japan) for study comparisons.

For the assessment of Cdk9 and cyclin T1 expression, the intensity of immunostaining was scored on a scale of 0 (no staining) to 4 (strongest intensity), and the percentage of cell staining at each intensity was estimated from 0 to 100. The percentage of cells at each intensity level was multiplied by the corresponding intensity value, and these products were combined to obtain an immunostaining score ranging from 0 to 400.

Statistical analysis. The disease-free survival (DFS) rates of patients were analyzed by Kaplan-Meier estimates and compared using the log-rank test. The DFS was defined as the interval between the date of surgery and the date of tumor recurrence or distant metastasis. Cox regression methods were used to investigate the association between survival, clinical parameters and immunohistochemical variables in multivariate models. Differences between positive and negative cyclin T1 staining were analyzed using the Mann-Whitney U-test. All the statistical tests were two-sided. The difference between the groups was considered statistically significant when $P<0.05$. All the analyses were performed using the SPSS 16.0 (SPSS, Inc., Chicago, IL, USA) software package.

Results

Patient variables. A total of 30 patients were examined in the study (15 male and 15 female). The mean age was 60.3 years; 14 patients were <60 years old and 16 were >60. Eleven patients developed tumor recurrence or distant metastases during the study. Fifteen of the patients who were at high risk of recurrence according to the NIH consensus received postoperative adjuvant chemotherapy with imatinib, and 1 patient received sunitinib. The other 14 patients did not receive postoperative adjuvant chemotherapy. The scores of Ki67 staining for 6 and 8 patients were > and <5%, respectively. The results of the other 16 patients were not recorded.

Ten patients were cyclin T1-positive (Fig. 1), and 20 were negative. All 11 patients with recurrent tumors or distant metastases were cyclin T1-negative patients. The DFS was analyzed using univariate log-rank test and multivariate stepwise Cox-regression test. Old age, large tumor size, higher Ki67 staining, high mitotic count and negative cyclin T1 staining were associated with clinical outcomes in univariate analysis. The Kaplan-Meier survival curve illustrated the DFS of the patients with negative cyclin T1 staining was significantly lower than that of the patients with positive cyclin T1 staining ($P=0.031$, Fig. 2). However, no significant difference was observed in the DFS between different ages, genders, tumor size, pathological type, Ki67 staining and cyclin T1 staining using the multivariate analysis. The clinicopathological features and univariate and multivariate analyses are summarized in Table II.

Table I. Antibodies used in the present study.

Antigen	Clone	Product code	Antibody class	Supplier	Dilution	Antigen Retrieval, min
CyclinT1	Rabbit polyclonal	ab2098	IgG	Abcam	1:500	ER1 20
Cdk9	Rabbit monoclonal	2454-1	IgG	Epitomics	1:500	ER2 20

ER1: Bond epitope retrieval solution 1 contains a citrate-based buffer and surfactant. ER2: Bond epitope retrieval solution 2 contains an ethylene diamine-tetra-acetic acid-based buffer and surfactant. IgG, immunoglobulin G.

Table II. Univariate log-rank and multivariate Cox analyses for prognostic factors with respect to disease-free survival.

			P-value	
Parameters	Cases, n	Events, n	Univariate	Multivariate
Gender				
Male	15	5	0.404	
Female	15	6		
Age, years				
≤60	14	2	0.026[a]	0.765
>60	16	9		
Type				
Spindle	26	9	0.088	0.834
Epitheloid/ mixed	4	2		
Tumor size, cm				
≤5	11	2	0.023[a]	0.959
>5	19	9		
Ki67				
≤5[b]	8	0	0.018[a]	0.675
>5[b]	6	3		
Mitosis, HPF				
≤5/50	23	6	0.021[a]	0.894
>5/50	7	5		
Cdk9				
≤270[b]	14	5	0.928	
>270[b]	16	6		
Cyclin T1				
-	20	11	0.031[a]	0.874
+	10	0		

[a]Statistically significant; [b]total score. HPF, high-power field.

Table III. Association of cyclin T1 with various clinicopathological parameters.

		Cyclin T1		
Parameters	Cases, n	-	+	P-value
Age, years				
≤60	14	8	6	0.442
>60	16	12	4	
Gender				
Male	15	9	6	0.699
Female	15	11	4	
Tumor size, cm				
≤5	11	7	4	>0.9999
>5	19	13	6	
Type				
Spindle	26	17	9	>0.9999
Epitheloid/mixed	4	3	1	
Ki67				
≤5%	8	5	3	>0.9999
>5%	6	4	2	
Mitosis, HPF				
≤5/50	23	13	10	0.064
>5/50	7	7	0	
Cdk9				
≤270	14	10	4	0.709
>270	16	10	6	

HPF, high-power field.

Subgroup analysis for cyclin T1. The patients were also divided into two subgroups on the basis of positive and negative staining for cyclin T1. Cyclin T1 staining was analyzed with the other clinicopathological factors. No significant differences were observed between the two subgroups. However, patients with negative cyclin T1 staining appeared to have poorer results than those with positive cyclin T1 staining. Among the 30 patients, 23 patients had low mitotic rates, and 7 had high rates. All 7 patients with high mitotic rates were cyclin T1-negative. A summary of the association between cyclin T1 staining and other clinicopathological factors are shown in Table III.

Discussion

In the present study, age, tumor size, percentage of Ki-67 staining, mitotic rate and cyclin T1 staining were the prognostic factors associated with recurrence in patients with gastric GIST following surgical resection. The results showed a significant difference in the univariate analysis but not in the multivariate analysis. Along with the factors of large tumor size and high mitotic rate presented in the NIH consensus, several studies have defined other poor prognostic factors,

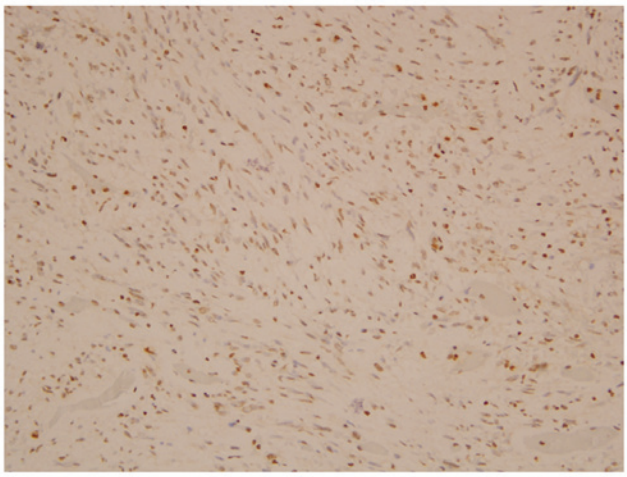

Figure 1. Positive cyclin T1 staining.

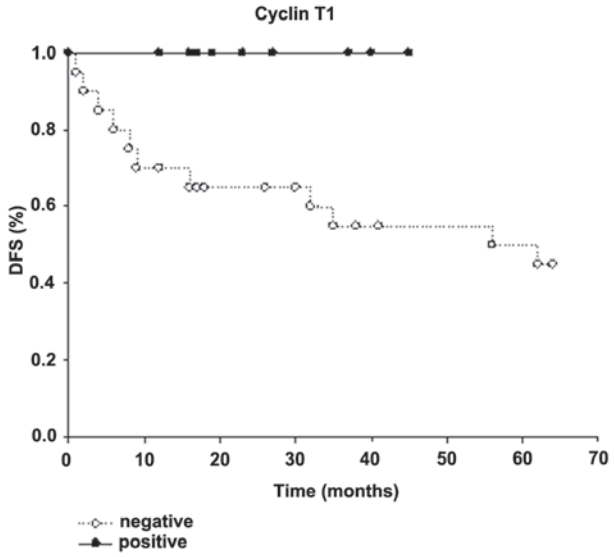

Figure 2. Kaplan-Meier survival curve of patients with gastrointestinal stromal tumors with negative or positive cyclin T1 staining. Patients with positive staining for cyclin T1 (n=30) showed a significantly good disease-free survival rate (P=0.031).

such as epithelioid type, increased expression of cytoplasmic HuR and cyclin A, and a high Ki67 ratio (24-27). Increased expression of cyclin H was also a poor prognostic factor in high-risk GIST patients (23). The present results showed that cyclin T1 may be another potential prognostic predictor for gastric GIST patients.

The association between cyclin T1 staining and other factors was also analyzed. Although no statistical significance was observed in the difference in mitotic rates between the cyclin T1-positive and -negative patients, all 10 patients with positive cyclin T1 staining had low mitotic rates, and all 7 patients with high mitotic rates were negative for cyclin T1. Cyclin T1 may be a potential regulator of mitosis and may contribute to tumor recurrence.

In a previous study, Cdk9/cyclin T1 complex upregulation contributed to T lymphocyte differentiation and malignant transformation (28). Regulation of the Cdk9/cyclin T1 complex is dependent on a tissue-specific signaling pathway (29), and the complex response to certain cytokines such as tumor necrosis factor and interleukin-6 (30,31). Cyclin T1 and Cdk9 may promote the expression of anti-apoptotic factors and cause proliferation (32-34). A deregulated Cdk9-related pathway has been observed in several human tumors, including lymphoma (29,35,36), neuroblastoma (37), prostate cancer (38) and several hematopoietic malignancies (29). By contrast, certain studies have demonstrated that cyclin T1, but not Cdk9, induced transformation *in vitro* in head and neck tumors (10). Upregulation of cyclin T1 is the main mechanism for activation of the complex during T-cell activation, and cyclin T1 acts as a rate-limiting regulatory subunit (39). A previous study also suggested that the cyclin-dependent kinase inhibitor had no correlation with the malignant potential of GIST and did not serve as a predictor of DFS (40). The present results demonstrated that cyclin T1, but not Cdk9, was a prognostic factor for DFS, and that cyclin T1 was associated with the mitotic rate. The study confirmed that cyclin T1 has a regulatory role in the Cdk9/cyclin T complex, and that upregulation of cyclin T1 was the main mechanism for the activation of this complex. These results also supported that cyclin T1 acted as a rate-limited regulatory subunit (39).

Certain previous studies have shown that cyclin T1 overexpression was a poor prognostic factor (10,28,29,39). However, the present results demonstrated that negative staining for cyclin T1 was a poor prognostic factor. Cdk9 and cyclin T1 were expressed in a similar pattern in certain normal tissues, but varied in other tissues (12). The tissues of mesenchymal organs, such as connective tissue, skeletal muscle, blood and lymphoid tissue, exhibited high cyclin T1 expression levels (11,12). It is also believed that cyclin T1 is not a typical cell cycle regulator, as its levels do not oscillate at any phase during the cell cycle (11). Additionally, the upregulation of cyclin T1 is not linked directly to cell cycle entry and progression (11). In different tissues, the expression of cyclin T1 has different roles in tumor behavior. Deregulation of cyclin T1 contributes to poor outcomes, and negative cyclin T1 expression is potentially associated with a worse prognosis.

The present study had several limitations. The subgroups of patients were too small for individual analysis. A study with a larger sample size will be necessary for further investigation of the predictors of gastric GISTs. In addition, *in vitro* cell molecular studies would help to identify the pathway of cyclin T1. This was a pilot study to determine whether cyclin T1 could be considered for further studies including more patients and cell lines to confirm the role of the Cdk9/cyclin T1 complex in GIST.

In conclusion, it is reasonable to consider cyclin T1 immunohistochemical staining as a predictor of the prognosis of gastric GIST following surgical resection. The pathway of cyclin T1 was also demonstrated to potentially be associated with the mitotic rate.

Acknowledgements

The present study was supported by a grant from Tungs' Taichung MetroHarbor Hospital (no. TTMHH-100R0004).

References

1. Scarpa M, Bertin M, Ruffolo C, Polese L, D'Amico DF and Angriman I: A systemic review on the clinical diagnosis of gastrointestinal stromal tumors. J Surg Oncol 98: 384-392, 2008.
2. Miettinen M, Sarlomo-Rikala M and Lasota J: Gastrointestinal stromal tumors: Recent advances in understanding of their biology. Hum Pathol 30: 1213-1220, 1999.
3. Fletcher CD, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, Miettinen M, O'Leary TJ, Remotti H, Rubin BP, *et al*: Diagnosis of gastrointestinal stromal tumors: A consensus approach. Int J Surg pathol 10: 81-99, 2002.
4. Huang HY, Li CF, Huang WW, Hu TH, Lin CN, Uen YH, Hsiung CY and Lu D: A modification of NIH consensus criteria to better distinguish the highly lethal subset of primary localized gastrointestinal stromal tumors: A subdivision of the original high-risk group on the basis of outcome. Surgery 141: 748-756, 2007.
5. Demetri GD, von Mehren M, Antonescu CR, DeMatteo RP, Ganjoo KN, Maki RG, Pisters PW, Raut CP, Riedel RF, Schuetze S, *et al*: NCCN task force report: Update on the management of patients with gastrointestinal stromal tumors. J Natl Compr Canc Netw 8 (Suppl 2): S1-S41, 2010.
6. Joensuu H: Risk stratification of patients diagnosed with gastro-intestinal stromal tumor. Hum Pathol 39: 1411-1419, 2008.
7. Miettinen M and Lasota J: Gastrointestinal stromal tumors-definition, clinical, histological, immunohistochemical and molecular genetic features and differential diagnosis. Virchows Arch 438: 1-12, 2001.
8. Miettinen M, Sobin LH and Lasota J: Gastrointestinal stromal tumors of the stomach: A clinicopathologic, immunohistochemical and molecular genetic study of 1765 cases with long-term follow-up. Am J Surg Pathol 29: 52-68, 2005.
9. Miettinen M, Makhlouf H, Sobin LH and Lasota J: Gastrointestinal stromal tumors of the jejunum and ileum: A clinicopathologic, immunohistochemical and molecular genetic study of 906 cases before imatinib with long-term follow-up. Am J Surg Pathol 30: 477-489, 2006.
10. Moiola C, De Luca P, Gardner K, Vazquez E and De Siervi A: Cyclin T1 overexpression induces malignant transformation and tumor growth. Cell Cycle 9: 3119-3126, 2010.
11. De Luca A, De Falco M, Baldi A and Paggi MG: Cyclin T: Three forms for different roles in physiological and pathological functions. J Cell Physiol 194: 101-107, 2003.
12. De Luca A, Russo P, Severino A, Baldi A, Battista T, Cavallotti I, De Luca L, Baldi F, Giordano A and Paggi MG: Pattern of expression of cyclin T1 in human tissues. J Histochem Cytochem 49: 685-692, 2001.
13. Sano M and Schneider MD: Cyclin-dependent kinase-9: An RNAPII kinase at the nexus of cardiac growth and death cascades. Circ Res 95: 867-876, 2004.
14. Sano M, Wang SC, Shirai M, Scaglia F, Xie M, Sakai S, Tanaka T, Kulkarni PA, Barger PM, Youker KA, *et al*: Activation of cardiac Cdk9 represses PGC-1 and confers a predisposition to heart failure. EMBO J 23: 3559-3569, 2004.
15. Chiu YL, Cao H, Jacque JM, Stevenson M and Rana TM: Inhibition of human immunodeficiency virus type 1 replication by RNA interference directed against human transcription elongation factor P-TEFb (CDK9/CyclinT1). J Virol 78: 2517-2529, 2004.
16. Chao SH and Price DH: Flavopiridol inactivates P-TEFb and blocks most RNA polymerase II transcription in vivo. J Biol Chem 276: 31793-31799, 2001.
17. Napolitano G, Majello B and Lania L: Role of cyclinT/Cdk9 complex in basal and regulated transcription (review). Int J Oncol 21: 171-177, 2002.
18. Chen R, Keating MJ, Gandhi V and Plunkett W: Transcription inhibition by flavopiridol: Mechanism of chronic lymphocytic leukemia cell death. Blood 106: 2513-2519, 2005.
19. Shan B, Zhuo Y, Chin D, Morris CA, Morris GF and Lasky JA: Cyclin-dependent kinase 9 is required for tumor necrosis factor-alpha-stimulated matrix metalloproteinase-9 expression in human lung adenocarcinoma cells. J Biol Chem 280: 1103-1111, 2005.
20. Nemoto Y, Mikami T, Hana K, Kikuchi S, Kobayashi N, Watanabe M and Okayasu I: Correlation of enhanced cell turnover with prognosis of gastrointestinal stromal tumors of the stomach: relevance of cellularity and p27kip1. Pathol Int 56: 724-731, 2006.
21. Liu FY, Qi JP, Xu FL and Wu AP: Clinicopathological and immunohistochemical analysis of gastrointestinal stromal tumor. World J Gastroenterol 12: 4161-4165, 2006.
22. Koon N, Schneider-Stock R, Sarlomo-Rikala M, Lasota J, Smolkin M, Petroni G, Zaika A, Boltze C, Meyer F, Andersson L, *et al*: Molecular targets for tumour progression in gastrointestinal stromal tumours. Gut 53: 235-240, 2004.
23. Dorn J, Spatz H, Schmieder M, Barth TF, Blatz A, Henne-Bruns D, Knippschild U and Kramer K: Cyclin H expression is increased in GIST with very-high risk of malignancy. BMC Cancer 10: 350, 2010.
24. Wei YC, Chou FF, Li CF, Li WM, Chen YY, Lan J, Li SH, Fang FM, Hu TH, Yu SC, *et al*: HuR cytoplasmic expression is associated with increased cyclin A expression and inferior disease-free survival in patients with gastrointestinal stromal tumours (GISTs). Histopathology 63: 445-454, 2013.
25. Rubin BP: Gastrointestinal stromal tumours: An update. Histopathology 48: 83-96, 2006.
26. Wong NA: Gastrointestinal stromal tumours-an update for histopathologists. Histopathology 59: 807-821, 2011.
27. ESMO/European Sarcoma Network Working Group: Gastrointestinal stromal tumors: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 23 (Suppl 7): vii, S49-S55, 2012.
28. Leucci E, De Falco G, Onnis A, Cerino G, Cocco M, Luzzi A, Crupi D, Tigli C, Bellan C, Tosi P, *et al*: The role of the Cdk9/cyclin T1 complex in T cell differentiation. J Cell Physiol 212: 411-415, 2007.
29. Bellan C, De Falco G, Lazzi S, Micheli P, Vicidomini S, Schürfeld K, Amato T, Palumbo A, Bagella L, Sabattini E, *et al*: CDK9/CYCLIN T1 expression during normal lymphoid differentiation and malignant transformation. J Pathol 203: 946-952, 2004.
30. De Luca A, Russo P, Severino A, Baldi A, Battista T, Cavallotti I, De Luca L, Baldi F, Giordano A and Paggi MG: Pattern of expression of cyclin T1 in human tissues. J Histochem Cytochem 49: 685-692, 2001.
31. MacLachlan TK, Sang N, De Luca A, Puri PL, Levrero M and Giordano A: Binding of CDK9 to TRAF2. J Cell Biochem 71: 467-478 1998.
32. Peng J, Zhu Y, Milton JT and Price DH: Identification of multiple cyclin subunits of human P-TEFb. Genes Dev 12: 755-762, 1998.
33. Napolitano G, Majello B, Licciardo P, Giordano A and Lania L: Transcriptional activity of positive transcrip-tion elongation factor b kinase in vivo requires the C-terminal domain of RNA polymerase II. Gene 254: 139-145, 2000.
34. Chen R, Keating MJ, Gandhi V and Plunkett W: Transcription inhibition by flavopiridol: Mechanism of chronic lymphocytic leukemia cell death. Blood 106: 2513-2519, 2005.
35. Fu TJ, Peng J, Lee G, Price DH and Flores O: Cyclin K functions as a CDK9 regulatory subunit and partici-pates in RNA polymerase II transcription. J Biol Chem 274: 34527-34530, 1999.
36. Bettayeb K, Tirado OM, Marionneau-Lambot S, Ferandin Y, Lozach O, Morris JC, Mateo-Lozano S, Drueckes P, Schächtele C, Kubbutat MH, *et al*: Meriolins, a new class of cell death inducing kinase inhibitors with enhanced selectivity for cyclin-dependent kinases. Cancer Res 67: 8325-8334, 2007.
37. De Falco G and Giordano A: CDK9: From basal transcrip-tion to cancer and AIDS. Cancer Biol Ther 1: 342-347, 2002.
38. Lee DK, Duan HO and Chang C: Androgen receptor interacts with the positive elongation factor P-TEFb and enhances the efficiency of transcriptional elongation. J Biol Chem 276: 9978-9984, 2001.
39. Garriga J, Peng J, Parreño M, Price DH, Henderson EE and Graña X: Upregulation of cyclin T1/CDK9 complexes during T cell activation. Oncogene 17: 3093-3102, 1998.
40. Shirin H, Kravtsov V, Shahmurov M, Shabat VS, Krinshpon I, Alin A, Avinoach I and Avni Y: The cyclin-dependent kinase inhibitor, p27, has no correlation with the malignant potential of GIST. Digestion 75: 4-9, 2007.