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Increased cyclin T1 expression as a favorable prognostic factor in treating gastric adenocarcinoma

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Abstract. The expression of cyclin A, B1, D1 and E in gastric adenocarcinoma is known to be associated with clinical outcome. However, few studies have investigated the role of cyclin T1 and cyclin-dependent kinase 9 (CDK9) in gastric adenocarcinoma. Therefore, this study assessed the clinical significance of cyclin T1 and CDK9 expression in gastric adenocarcinoma. A total of 39 gastric adenocarcinoma patients received either radical total or distal gastrectomy in this study. Surgical tissue slides were stained with CDK9 and cyclin T1 antibodies, and immunohistochemistry scores and disease-free survival (DFS) rates were analyzed. Among the 19 patients with tumor-recurrent or distant metastasis, 16 were recorded as exhibiting low expression of cyclin T1. The remaining three patients exhibited high expression of the antibody. The results of patients with a higher T stage, N stage and tumor grade were less favorable. For patients with adenocarcinoma, the percentage of tissue slides stained with cyclin T1 was significantly higher than for those with normal stomach epithelia. The DFS rates of patients with low expression of cyclin T1 were significantly associated with poorer DFS rates. In conclusion, high expression of cyclin T1 is a favorable prognostic factor in treating patients with stomach adenocarcinoma.

Introduction

Gastric adenocarcinoma is a prevalent disease that accounts for ~10% of cancers worldwide (1). In recent decades, advances in surgical treatment, postoperative care and multimodality therapy have yielded modest improvements in the prognosis of the disease. Gastric adenocarcinoma is considered a heterogeneous disease exhibiting multiple epidemiological and histopathological characteristics (2). However, the uniform treatment of the disease disregards the histological subtypes of adenocarcinoma. The molecular study of gastric cancer may clarify the pathogenesis of tumors and facilitate the identification of alternative forms of effective treatment.

Positive transcriptional elongation factor b (P-TEFb) is a complex that contains the catalytic subunit, cyclin-dependent kinase 9 (CDK9), and the regulatory subunit, cyclin T. Cyclin T contains subunits T1, T2a and T2b. In general, CDK9 consists of a complex of T1, T2a and T2b at ratios of ~80%, 10% and 10%, respectively (3-5). The expression pattern of cyclin T2a almost completely overlaps the pattern described for cyclin T1 (5). The expression of cyclin T1 increases CDK9 activity and the phosphorylation of RNA polymerase II (RNAPII). The hypophosphorylated carboxyl-terminal domain of RNAPII instigates the elongation phase of transcription (6), thus enabling RNAPII to escape from promoter-proximal pausing factors in pre-mRNA processing (7).

P-TEFb has been thoroughly investigated in cardiac hypertrophy and HIV infection. Interaction with human cyclin T1 is required for the transcriptional activation of HIV-1. The interaction forms a complex with P-TEFb, which increases the RNAPII number (8). In addition, the CDK inhibitor, flavopiridol, was reported to exhibit certain therapeutic qualities (9). However, hypertrophic signals may activate CDK9 and the consequent phosphorylation of RNAPII. This effect not only increases RNA synthesis but also enlarges myocytes. This potentially results in cardiac hypertrophy (6,7). A previous study suggests that P-TEFb and transcriptional elongation play crucial roles in protecting normal and cancer cells from apoptosis (10). CDK9 inhibitors are generally considered potential therapeutic agents in chronic lymphocytic leukemia (11) and lung adenocarcinoma (12). Cyclin T1 and CDK9 are also overexpressed in the cell lines of human head and neck carcinoma (3).

Clinical studies have identified the expression of cyclin A, B1, D1 and E in gastric adenocarcinoma. Cyclin D1 and E

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Table I. Antibodies used in study.

Antigen	Clone	Product code	Antibody class	Supplier	Dilution	Antigen retrieval
Cyclin T1	Rabbit polyclonal	ab2098	IgG	Abcam	1:500	ER1 20 min
CDK9	Rabbit monoclonal	2454-1	IgG	Epitomics	1:500	ER2 20 min

CDK9, cyclin-dependent kinase 9; ER1, bond epitope retrieval solution 1 containing a citrate-based buffer and surfactant; ER2, bond epitope retrieval solution 2 containing an ethylene diaminetetra-acetic acid-based buffer and surfactant.

are the key regulators of progression through the G1 phase of the cell cycle (13,14). Overexpression of cyclin D1 and E is considered an early biomarkers of gastric cancer (15). The overexpression of cyclin E has been considered alternatively as a favorable, poor and irrelevant prognostic factor in clinical outcome (16). Cyclin A may be activated during the transition from the G1 to the S phase of the cell cycle (17,18). Cyclin B1 regulates cell progression through the G2 and M phase. Overexpression of cyclin B1 is associated with tumor progression and poor prognosis (19). However, there are few effective clinical predictors for gastric adenocarcinoma. This study, therefore, assessed the clinical significance of cyclin T1 and CDK9 expression in gastric adenocarcinoma.

Materials and methods

Tissues and patients. Tests were conducted on 39 gastric adenocarcinoma patients, all of whom had received either radical total or distal gastrectomy during the period from 2008 to 2011 from the same team at Tungs' Taichung Metro Harbor Hospital, Taiwan. Normal gastric tissues were collected from 16 other patients. Twenty-two of the patients received postoperative adjuvant chemotherapy. The patients' medical charts, pathological reports and surgical notes were retrospectively reviewed. The pathological diagnoses in these cases were reviewed by at least two experienced pathologists. The Tumor-Node-Metastasis (TNM) system was used according to the seventh edition of the AJCC cancer staging manual (20). As recognized in the 2010 World Health Organization classification, there are four major histological patterns of gastric cancers: tubular, papillary, mucinous and poorly differentiated signet ring cell carcinoma (21). The specimens were fixed in formalin and embedded in paraffin wax; the fixed paraffin was then cut into 3-μm sections.

Ethics. This study was approved by the Institutional Review Board of the Tungs' Taichung Metro Harbor Hospital (approval number 100006). All patients included in the study were informed of the involved procedures and provided written consent prior to the collection of all specimens and clinical information.

Immunohistochemical staining. Slides were stained with the CDK9 monoclonal antibody and the cyclin T1 polyclonal antibody using the Bond-Max autostainer (Leica Microsystems, Melbourne, Australia). Table I lists the details of these immunomarkers, including methods of pretreatment for antigen retrieval. In brief, formalin-fixed and paraffin-embedded tissue specimens were introduced to Tris-buffered saline and Tween-20. They were then rehydrated through serial dilutions of alcohol, before being washed in phosphate-buffered saline (pH 7.2). Coated slides were then stained with the aforementioned antibodies. This procedure was performed on the fully automated Bond-Max system using onboard heat-induced antigen retrieval and a Leica Refine polymer detection system (Leica Microsystems). Diaminobenzidine was used as the chromogen (Leica Microsystems) in all immunostainings. Negative controls were obtained by excluding the primary antibody, and appropriate positive controls were applied throughout the study. Finally, the slides were mounted with gum for examination, and the images of each slide were captured using the Olympus BX51 microscope/DP71 digital camera system (Ina-shi, Nagano, Japan) for comparison.

To assess CDK9 and cyclin T1 expression, the intensity of immunostaining was scored on a scale of 0 (no staining) to 4 (strongest intensity). The percentage of cell staining at each intensity level was estimated from 0 to 100. The percentage of cells at each intensity level was then multiplied by the corresponding intensity value, thus providing an immunostaining score ranging from 0 to 400.

Statistical analysis. Twenty-five patients with a cyclin T1 score ≤180 were defined as having low expression. Fourteen patients with cyclin T1 scores >180 were defined as having high expression. Seventeen patients with a CDK9 score ≤240 were defined as having low expression. Twenty-two patients with CDK9 scores >240 were defined as having high expression.

The disease-free survival (DFS) rates of patients were analyzed by applying Kaplan-Meier estimates and compared according to the log-rank test. The DFS rate 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 determine the correlation among survival, clinical parameters and immunohistochemical variables in multivariate models.

The differences between positive and negative cyclin T1 or CDK9 stains were analyzed using Mann-Whitney U-tests. All statistical tests were two-sided. $P<0.05$ was considered to indicate a statistically significant difference between groups.

The results of immunohistochemical staining intensity, the percentage of tumor staining and the total score are expressed as the mean ± standard errors of the mean. Statistical analysis was performed using a nonparametric Chi-square test, and $P<0.05$ was considered to indicate a statistically significant difference. All analyses were performed using the SPSS 16.0 software package (SPSS, Inc., Chicago, IL, USA) .

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

Parameters	Category	No. of cases	No. of events	P-value	
				Univariate	Multivariate
Gender	Male	25	14	0.244	
	Female	14	5		
Age	≤60	13	6	0.303	
	>60	26	13		
Cell type	Tubular	14	5	0.268	
	Papillary	7	4		
	Mucinous	3	1		
	Signet ring	15	9		
T stage	1	1	0	0.018[a]	0.581
	2	14	5		
	3	16	8		
	4	8	6		
N stage	0	7	2	0.040[a]	0.083
	1	13	7		
	2	8	2		
	3	11	8		
Grade	1	0	0	<0.0001[a]	0.007[a]
	2	12	6		
	3	23	9		
	4	4	4		
CDK9 score	≤240	17	10	0.549	
	>240	22	9		
Cyclin T1 score	≤180	25	16	0.028[a]	0.033[a]
	>180	14	3		

CDK9, cyclin-dependent kinase 9. [a]P<0.05.

Results

In this study, 39 patients were examined. The mean age was 67.4 years, and the group included 13 patients of 60 years old or less and 26 patients over 60 years old. Among the patients, 25 were male and 14 were female. A total of 19 patients developed tumor recurrence or distant metastasis during the study, and 22 received postoperative adjuvant chemotherapy. The other patients did not receive postoperative adjuvant chemotherapy.

Among the 19 patients with tumor-recurrent or distant metastasis, 16 exhibited low cyclin T1 expression (score ≤180) and only 3 exhibited high cyclin T1 expression (score >180) (Fig. 1). The DFS rates were analyzed using the univariate log-rank test and the multivariate stepwise Cox-regression test. Higher T stage, higher N stage, higher tumor grade and lower cyclin T1 scores in the univariate analysis revealed poorer clinical outcomes. The Kaplan-Meier survival curve demonstrated that the DFS rate of patients with low expression of cyclin T1 was significantly lower than that of patients with higher expression of cyclin T1 (P=0.028, Fig. 2). In addition, the differential DFS rate among T stage, N stage, tumor grade and cyclin T1 expression was significant, based on multivariate analysis. The clinicopathological features of the univariate and multivariate analyses are summarized in Table II. The cyclin T1 score was also analyzed in association with other clinicopathological factors. No significant difference was observed between the two subgroups, except with regard to the histological subtype. Tubular-type adenocarcinoma demonstrated a significantly higher cyclin T1 expression. A summary of the correlation between cyclin T1 staining scores and other clinicopathological factors is shown in Table III.

The immunostaining scores of cyclin T1 and CDK9 in gastric adenocarcinoma and normal gastric epithelia are shown in Tables IV and V. The staining percentages and total scores of cyclin T1, but not CDK9, were significantly higher in the adenocarcinoma group than in the normal stomach epithelia group. The immunostaining scores of cyclin T1 and CDK9 for the four histological subtypes of gastric adenocarcinoma are shown in Tables VI and VII. These results reveal that the intensity of cyclin T1 and CDK9 is higher in tubular-type stomach adenocarcinoma than in its papillary-type equivalent.

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

Parameters	Category	No. of cases	Cyclin T1 score		P-value
			≤180	>180	
Cell type	Tubular	14	4	10	0.004[a]
	Papillary	7	6	1	
	Mucinous	3	2	1	
	Signet ring	15	13	2	
Age	≤60	13	9	4	0.733
	>60	26	16	10	
Gender	Male	25	17	8	0.741
	Female	14	8	6	
T stage	1	1	1	0	0.302
	2	14	7	7	
	3	16	10	6	
	4	8	7	1	
N stage	0	7	4	3	0.967
	1	13	9	4	
	2	8	5	3	
	3	11	7	4	
Grade	1	0	0	0	0.544
	2	12	9	3	
	3	23	13	10	
	4	4	3	1	
CDK9 score	≤240	17	13	4	0.281
	>240	22	12	10	

CDK9, cyclin-dependent kinase 9. [a]P<0.05.

Table IV. Cyclin T1 immunostaining scores in gastric adenocarcinoma and normal tissue.

Tissue	Cyclin T1		
	Intensity	% staining	Total score
Adenocarcinoma (n=39)	2.00±1.17	73.72±27.57	158.72±114.76
Normal gastric epithelia (n=16)	1.19±1.11	17.50±18.80[a]	36.87±39.62[a]

Data are means ± standard error of the mean immunostaining score for cyclin T1 in gastric adenocarcinoma and normal tissue. [a]Significant difference in cyclin T1 expression between adenocarcinoma and normal gastric epithelia, P<0.05.

Table V. CDK9 immunostaining scores in gastric adenocarcinoma and normal tissue.

Tissue	CDK9		
	Intensity	% staining	Total score
Adenocarcinoma (n=39)	2.77±1.29	72.69±32.48	237.95±132.37
Normal gastric epithelia (n=16)	3.19±0.98	75.00±22.51	255.63±88.62

Data are means ± standard error of the mean immunostaining score for CDK9 in gastric adenocarcinoma and normal tissue. CDK9, cyclin-dependent kinase 9.

Table VI. Cyclin T1 immunostaining scores in gastric adenocarcinoma tissue.

	Cyclin T1		
Histological type	Intensity	% staining	Total score
Tubular (n=14)	2.57±0.94	83.21±24.78	220.00±108.63
Papillary (n=7)	1.29±0.76[a]	71.43±26.10	100.00±91.10
Mucinous (n=3)	1.67±0.58	66.67±35.12	123.33±86.22
Signet ring (n=15)	1.87±1.41	67.33±29.63	136.00±117.77

Data are means ± standard error of the mean immunostaining score for cyclin T1 in gastric adenocarcinoma. [a]Significant difference in cyclin T1 expression between tubular and papillary subtypes, P<0.05.

Table VII. CDK9 immunostaining scores in gastric adenocarcinoma.

	CDK9		
Histological type	Intensity	% staining	Total score
Tubular (n=14)	3.36±0.63	88.57±12.77	302.86±89.99
Papillary (n=7)	1.57±1.62[a]	50.00±47.61	141.43±155.40
Mucinous (n=3)	2.33±2.08	60.00±51.96	210.00±187.35
Signet ring (n=15)	2.87±1.13	71.00±28.42	228.00±124.63

Data are means ± standard error of the mean immunostaining score for CDK9 in gastric adenocarcinoma. CDK9, cyclin-dependent kinase 9. [a]Significant difference in CDK9 expression between tubular and papillary subtypes, P<0.05.

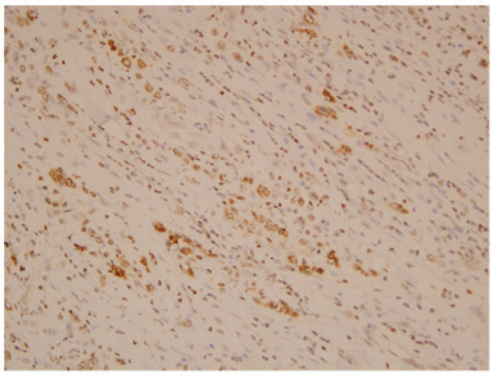

Figure 1. Positive cyclin T1 staining in a high-power field.

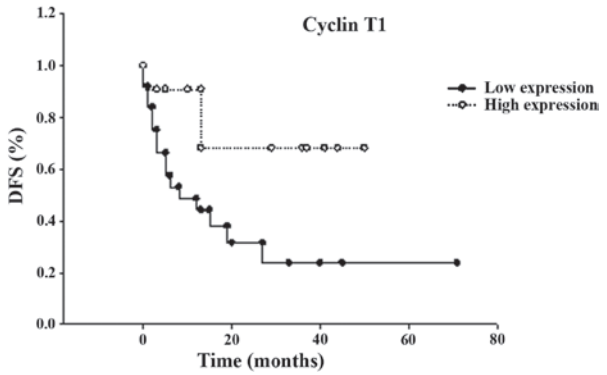

Figure 2. Kaplan-Meier survival curve of patients with stomach adenocarcinoma (n=39) with high (>180) or low (≤180) cyclin T1 scores. Increases in the disease-free survival (DFS) rates of patients exhibiting high expression of cyclin T1 demonstrate a positive prognosis (P=0.028).

Discussion

In this study, T stage, N stage, tumor grade and cyclin T1 staining score were identified as recurrent prognostic factors of postsurgical resection in gastric adenocarcinoma. Univariate and multivariate analysis results demonstrated a significant difference in DFS rates between patients with high and low expression of cyclin T1. Other than T stage, N stage and tumor grade, alternative negative prognostic factors, including overexpression of cyclin A, B1, D1 and E (13-16) were also observed.

The correlation between the cyclin T1 staining score and various other factors was also analyzed. The immunostaining score of tubular-type adenocarcinoma was noted to be significantly higher than that of other types of stomach adenocarcinoma.

A previous study has revealed that upregulation of the CDK9/cyclin T1 complex contributes to T lymphocyte differentiation and malignant transformation (22). Regulation of the CDK9/cyclin T1 complex depends on its tissue-specific signaling pathway (23) and its response to cytokines including tumor necrosis factor and interleukin-6 (5,24). Deregulated CDK9-related pathways were observed in several human tumors including lymphoma (23,25,26), neuroblastoma (27),

prostate cancer (28) and several instances of hematopoietic malignancy (23). These studies have suggested that cyclin T1 and CDK9 may promote the expression of anti-apoptotic factors and induce proliferation (11,29,30). By contrast, an alternative study has demonstrated that cyclin T1, but not CDK9, induces the *in vitro* transformation of head and neck tumors (3). Upregulation of cyclin T1 is the main mechanism for the activation of the complex during T cell activation, and cyclin T1 acts as a rate-limiting subunit (31). Furthermore, our results reveal that cyclin T1, but not CDK9, is a potential prognostic factor for improving the DFS rate of gastric adenocarcinoma. However, it is possible that it may also induce malignant transformation.

Our results confirm that cyclin T1 plays a regulatory role in the CDK9/cyclin T complex and that the upregulation of cyclin T1 is the main mechanism in the activation of this complex. Our results also support that cyclin T1 acts as a rate-limiting positive regulatory subunit (31).

In contrast to previous studies (3,17,18,31) that have suggested that the overexpression of cyclin T1 is a poor prognostic factor, our results indicate that low cyclin T1 expression is a poor prognostic factor in treating stomach adenocarcinoma. Patterns of CDK9 and cyclin T1 expression were observed to be similar in normal organ tissue, yet quite different in other tissues (5). Cyclin T1 was expressed in a wide variety of human tissues. The tissue of mesenchymal organs, including connective tissue, skeletal muscle, blood and lymphoid tissue, exhibits high levels of cyclin T1 expression (4,5). In addition, cyclin T1 is not considered a typical cell cycle regulator as its levels do not oscillate in any phase during the cell cycle (4). Moreover, upregulation of cyclin T1 has not been linked directly to cell cycle entry or progression (4). In different tissues, the expression of cyclin T1 plays a different role in tumor behavior. Deregulation of cyclin T1 contributes to a poor outcome, and negative cyclin T1 expression is a potentially less favorable factor.

There were limitations to this study. The subgroups of patients were too small for individual subtype analysis. A study with a larger sample size is necessary for further differentiation of the predictors. In addition, *in vitro* cell molecular studies would have enabled identification of the cyclin T1 pathway. 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 gastric adenocarcinoma.

In conclusion, the results of this study confirm the hypothesis that high expression of cyclin T1 is a favorable prognostic factor in stomach adenocarcinoma.

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References

1. Ferlay J, Bray F, Pisani P and Parkin M: GLOBOCAN 2002: cancer incidence, mortality and prevalence worldwide. IARC Press, Lyon, 2004.
2. Shah MA, Khanin R, Tang L, Janjigian YY, Klimstra DS, Gerdes H and Kelsen DP: Molecular classification of gastric cancer: a new paradigm. Clin Cancer Res 17: 2693-2701, 2011.
3. 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.
4. 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.
5. 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.
6. 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.
7. 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.
8. 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.
9. 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.
10. 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.
11. 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.
12. 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: 1103-1011, 2005.
13. Sugai T, Tsukahara M, Endoh M, Shioi Y, Takebe N, Mue Y, Matsushita H, Toyota M and Suzuki K: Analysis of cell cycle-related proteins in gastric intramucosal differentiated-type cancers based on mucin phenotypes: a novel hypothesis of early gastric carcinogenesis based on mucin phenotype. BMC Gastroenterol 10: 55, 2010.
14. Ioachim E: Expression patterns of cyclins D1, E and cyclin-dependent kinase inhibitors p21waf1/cip1, p27kip1 in colorectal carcinoma: correlation with other cell cycle regulators (pRb, p53 and Ki-67 and PCNA) and clinicopathological features. Int J Clin Pract 62: 1736-1743, 2008.
15. Motohashi M, Wakui S, Muto T, Suzuki Y, Shirai M, Takahashi H and Hano H: Cyclin D1/cdk4, estrogen receptors α and β, in N-methyl-N'-nitro-N-nitrosoguanidine-induced rat gastric carcinogenesis: immunohistochemical study. J Toxicol Sci 36: 373-378, 2011.
16. Kouraklis G, Katsoulis IE, Theocharis S, Tsourouflis G, Xipolitas N, Glinavou A, Sioka C and Kostakis A: Does the expression of cyclin E, pRb and p21 correlate with prognosis in gastric adenocarcinoma? Dig Dis Sci 54: 1015-1020, 2009.
17. Li JQ, Miki H, Wu F, Saoo K, Nishioka M, Ohmori M and Imaida K: Cyclin A correlates with carcinogenesis and metastasis and p27(kip1) correlates with lymphatic invasion, in colorectal neoplasms. Hum Pathol 33: 1006-1015, 2002.
18. Mrena J, Wiksten JP, Kokkola A, Nordling S, Haglund C and Ristimäki A: Prognostic significance of cyclin A in gastric cancer. Int J Cancer 119: 1897-1901, 2006.
19. Yasuda M, Takesue F, Inutsuka S, Honda M, Nozoe T and Korenaga D: Overexpression of cyclin B1 in gastric cancer and its clinicopathological significance: an immunohistological study. J Cancer Res Clin Oncol 128: 412-416, 2002.
20. Washington K: 7th edition of the AJCC cancer staging manual: Stomach. Ann Surg Oncol 17: 3077-3079, 2010.
21. Berlth F, Bollschweiler E, Drebber U, Hoelscher AH and Moenig S: Pathohistological classification systems in gastric cancer: Diagnostic relevance and prognostic value. World J Gastroenterol 20: 5679-5684, 2014.
22. 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.
23. 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.

24. 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.
25. Fu TJ, Peng J, Lee G, Price DH and Flores O: Cyclin K functions as a CDK9 regulatory subunit and participates in RNA polymerase II transcription. J Biol Chem 274: 34527-34530, 1999.
26. 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.
27. De Falco G and Giordano A: CDK9: From basal transcription to cancer and AIDS. Cancer Biol Ther 1: 342-377, 2002.
28. 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.
29. Peng J, Zhu Y, Milton JT and Price DH: Identification of multiple cyclin subunits of human P-TEFb. Genes Dev 12: 755-762, 1998.
30. Napolitano G, Majello B, Licciardo P, Giordano A and Lania L: Transcriptional activity of positive transcription elongation factor b kinase in vivo requires the C-terminal domain of RNA polymerase II. Gene 254: 139-145, 2000.
31. 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.