

Combined Evaluation of Expressions of Cyclin E and p53 Proteins as Prognostic Factors for Patients with Gastric Cancer

Kamal E. Bani-Hani,¹ Nidal M. Almasri,²
Yousef S. Khader,³ Fawzi M. Sheyab,⁴ and
Hanan N. Karam⁴

Departments of ¹Surgery, ²Pathology, ³Public Health, and ⁴Molecular Biology, King Abdullah University Hospital, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan

ABSTRACT

Background: There is a lack of consistency regarding the prognostic value of cyclin E overexpression in gastric cancer (GC). Our aim was to report on this overexpression and to analyze its correlations with the clinicopathologic variables. Another aim was to examine if aberrant expression of both cyclin E and p53 might increase the malignant potential of gastric cancer.

Methods: Specimens from 89 patients with gastric cancer treated with “curative” intent were evaluated for cyclin E and p53 expressions using immunohistochemical method. The correlations between cyclin E overexpression alone or in combination with p53 expression and the patient’s clinicopathologic variables were analyzed.

Results: Cyclin E overexpression and p53 expression were shown in 35 (39.3%) and 46 (51.7%) tumors, respectively. The incidence of cyclin E overexpression was significantly higher in deeply invasive cancers ($P < 0.0001$), in cancers with lymph node metastasis ($P = 0.003$), and in cancers with advanced stages ($P < 0.0001$). There were no significant correlations with other clinicopathologic variables. Patients in whom their tumors showed cyclin E overexpression alone or in combination with p53 survived less than patients with negative cyclin E tumors. Multivariate analysis revealed that combined cyclin E overexpression and p53 expression was significantly associated with poor survival after adjusting for other variables (hazard ratio, 3.12; $P = 0.009$).

Conclusions: Cyclin E overexpression is a common event in gastric cancer. Gastric cancer with cyclin E overexpression exhibit increased aggressiveness in the presence of aberrant p53. The combination of cyclin E

overexpression with the p53 expression in gastric cancer further distinguished a subgroup of patients with poor prognosis.

INTRODUCTION

Gastric cancer is one of the major causes of cancer death worldwide (1). Its pattern and incidence vary widely between different parts of the world. Costa Rica and Japan have the first and second highest rates in the world with a death rate of 77.5 and 50.5 per 100,000 population, respectively (1, 2). In the United States, 12,100 deaths from gastric cancer were expected during 2003 with a death rate of 6.8 per 100,000 (3). The overall age-adjusted incidence (world population) of gastric cancer in Jordan is 5.82 per 100,000 per year (4). The risk of developing gastric cancer is relatively lower in the Middle East and North Africa compared with those of western countries and Japan (1, 4). Environmental factors such as *Helicobacter pylori* and dietary factors play an important role in gastric carcinogenesis (5, 6).

The molecular biology of gastric cancer has been widely studied in the developed world particularly in Japan (7–11). However, there are no similar published reports from the developing countries. It is established now that multiple and cumulative series of structural and functional genetic alterations of oncogenes, tumor suppressor genes, DNA repair genes, cell cycle regulators, cell adhesion molecules, and growth factors/receptors system, in addition to, genetic instability of other genetic foci are implicated in the development and progression of the multistep gastric carcinogenesis (7–11).

Cyclin E is a 395-amino acid protein derived from a gene on chromosome 19q12->q13 (12, 13). It is one of the most important cell cycle regulators that play an important role in normal cell proliferation and development through promotion of the S phase. As a late G₁ cyclin, it functionally form a complex with its catalytic subunit, the cyclin-dependent kinase 2, which is required for control of the G₁-S phase transition (14, 15). This cyclin E/cyclin-dependent kinase 2 complex leads to phosphorylation of key substrates essential for DNA synthesis. Cyclin E alterations, such as untimely and aberrant expression can cause deregulation of the cell cycle by speeding the G₁ phase and thus contributes to carcinogenesis and malignant progression through subsequent unbridled cellular growth and proliferation (7, 16). Cyclin E deregulations are general events in cancer, irrespective of the tumor origin. Amplification and/or overexpression of cyclin E have been observed in >48 malignancies, including cancers of the breast, colon, ovary, uterine cervix, endometrium, skin, adrenal cortex, kidney, and lung, as well as sarcomas and hematologic malignancies (17–27). These changes have been also reported in esophageal and gastric cancers (28–49).

In gastric cancer, the role of cyclin E as a prognosticator is controversial. Most reports showed that tumors with high cyclin E expression had a worse prognosis than those with low level of cyclin E, and these studies correlated high cyclin E expression

Received 8/26/04; revised 11/5/04; accepted 11/11/04.

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Requests for reprints: Kamal E. Bani-Hani, Department of Surgery, Faculty of Medicine, King Abdullah University Hospital, Jordan University of Science and Technology, P.O. Box 3030 Irbid, 22110, Jordan. Phone: 962-79-5500014; Fax: 962-2-7060300; E-mail: banihani60@yahoo.com.

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with tumor invasion, lymph node metastasis and poor prognosis (28–35, 45), whereas a fewer reports suggested that cyclin E is not a significant prognostic factor (36–39, 46). Yet another study (39) has shown that tumors with high cyclin E expression had a favorable prognosis than those with low level of cyclin E. Thus, there is a lack of consistency with regard to the role of cyclin E as a prognosticator in gastric cancer. The aim for this study was to examine the prognostic value of cyclin E overexpression in gastric cancer. Another aim for this study was to examine if aberrant expression of both cyclin E and p53, which act in the same pathway, might increase the malignant potential of gastric cancer.

MATERIALS AND METHODS

Tissue Samples and Patient Population. Tissues from surgical resection specimens of gastric cancer were obtained for each of the 89 patients who had potentially curative resection of their gastric cancer between 1991 and 2002 at King Abdullah University Hospital and its related facilities, Irbid-northern Jordan. The medical and pathologic reports of these patients were examined in details for age and gender of the patients. The tumor site, histologic subtype, grade, depth of penetration, lymph node status, and tumor stages, in addition to the presence of *H. pylori* infection and patient survival were analyzed. Data regarding tumor size were incomplete and were not included.

The whole group consisted of 59 males and 30 females (mean age, 58.5 years; range, 24–82 years). All patients underwent total or subtotal gastrectomy. None of the patients received adjuvant chemotherapy. Gastric cancer was classified according to the modified Lauren criteria (50). There were 59 tumors of intestinal type and 30 of diffuse type. The staging of gastric cancer was made in accordance with the tumor-node-metastasis (TNM) system endorsed by the Union Internationale Contre Le Cancer (51).

Vital status of patients was ascertained from death certificates or from the patients or their families who were contacted. Patients were followed until the earliest of the following: their date of death, the date they were last known to be alive, or the end of the follow-up period on 31st December 2003. Observations were censored at either the date of last known follow-up or the end date of the follow-up period if death had not occurred. Survival was calculated from the day gastrectomy was done to the day of death or the day of the last follow-up. The mean follow-up was 33.7 months (SD \pm 23.9; range, 1–132 months).

Immunohistochemical Staining. Anti-human cyclin E mouse monoclonal antibody and anti-p53 polyclonal antibody were purchased from Novocastra Laboratories Ltd. (Newcastle upon Tyne, United Kingdom). Sections (4 μ m) were cut from formalin-fixed, paraffin-embedded resected gastric cancer tissue samples and immunostaining was done following previously described methods (29, 34). Sections were deparaffinized in xylene and then rehydrated and equilibrated in TBS [50 mmol/L Tris and 145 mmol/L NaCl (pH 7.6)]. Sections were treated with 3% hydrogen peroxide in methanol for 30 minutes to remove endogenous peroxidase activity. The sections were placed in containers filled with 1,500 mL of 10 mmol/L sodium citrate buffer at pH 6 and then were heated in a microwave for 10 minutes to retrieve the antigens. To block nonspecific protein binding, we incubated sections for 10 minutes at room temperature in 20% normal goat serum. Nonspecific binding reactions were further

blocked by incubating the sections with an avidin-biotin blocking kit (Vector Laboratories Ltd., Burlingame, CA) according to the manufacturer's instructions prior to the application of the primary antibodies. Sections were incubated (2 hours at room temperature) with anti-cyclin E antibody (diluted 1:50) or anti-p53 antibody (diluted 1:1,000). Sections were then washed in TBS and incubated with biotinylated anti-mouse or rabbit IgG horse serum (Vector BA-2000; Vector Laboratories; diluted 1:100 in TBS) for 30 minutes at room temperature. Detection of bound secondary antibody was done using a Vectastain Elite avidin-biotin complex kit (Vector Laboratories) in conjunction with the chromogen 3,3'-diaminobenzidine according to the manufacturer's instructions. Throughout the study, sections from breast cancer tissues known to express cyclin E protein were analyzed in parallel to serve as positive controls. Omission of the primary antibody from these samples acted as a negative control. Histologically normal gastric tissue from the resections away from the tumor provided additional negative internal control for the cyclin E protein expression. All sections were counterstained with hematoxylin and examined by light microscopy. Each experiment was independently done twice.

Histologic and Immunohistochemical Assessment. All slides were coded and evaluated in a blinded fashion without knowledge of patient identity or clinical status by an experienced pathologist (N. Al-masri). Staining was evaluated in the adjacent normal gastric epithelial and tumor cells. The result of immunostaining was considered to be positive only if nuclear staining was observed. Cytoplasmic staining without nuclear positivity was regarded as negative. Intranuclear staining was graded semi quantitatively as described by Yasui et al. (29). Briefly, the immunoreactivity was scored from – to +++ according to the number of cells stained and the intensity of the reaction in individual cells. Scores were defined as follows: For cyclin E, score 0: negative or <5% nuclear or cytoplasmic stain; score 1: +, 5% to 25% of tumor cells showed weak to moderate immunoreactivity; score 2: ++, 26% to 50% of tumor cells showed moderate immunoreactivity or 10% to 50% of tumor cells showed intense immunoreactivity; score 3: +++, >50% of tumor cells showed intense immunoreactivity. As in the study by Yasui et al. (29), we regarded strong positive staining (score 2 or 3) as overexpression of cyclin E. For the purpose of statistical analysis we combined score 0 and 1 (negative and weakly positive) and score 2 and 3 (strongly positive). For p53, negative, almost no positive cells; +, 5% to 25% of tumor cells showed immunoreactivity; ++, 26% to 50% of tumor cells showed immunoreactivity; +++, >50% of tumor cells showed immunoreactivity.

In general, expression of cyclin E was heterogeneously distributed in most tumors. There were intratumoral as well as intertumoral differences in the expression of cyclin E, ranging from 0% up to a maximum of 85% cyclin E-positive tumor cells in an individual tumor. Thus, as in the study of Jiaqing et al. (32), we selected five areas (upper, center, and transverse invasive fronts and vertical invasive front bilateral of tumor) from each cancer and counted the number of stained cells per 200 cells for each area. A total of 1,000 cells were counted for each cancer and the average percentage of stained cells was calculated.

Morphologic examination for the presence of *H. pylori* was evaluated on the H&E-stained sections and confirmed by modified Giemsa stain when necessary. The results

of cyclin E expression were analyzed and correlated with the clinicopathologic data as well as with the expression of p53 to elucidate the role of cyclin E in the progression of gastric cancer.

Statistical Analysis. The frequency of cyclin E overexpression was compared with the clinicopathologic variables of the patients using Pearson's χ^2 test. The survival rate was analyzed by the Kaplan-Meier method, and the survival curves were stratified according to cyclin E and p53 expressions status by the log-rank test. Univariate analysis and multivariate Cox regression analysis were done to identify prognostic factors for survival. A group of patients with combined cyclin E overexpression and p53 expression was compared with other groups of patients that were combined because they had similar survival. To avoid sparseness that results from small cell counts, the variables (site, stage, and depth of penetration) were dicotomized. In addition, stepwise selection method with entry testing based on the significance of the score statistic and removal testing based on the probability of a likelihood-ratio statistic based on conditional variable estimates was used.

All analyses were done using the Statistical Package for the Social Sciences Software Program version 11 (SPSS, Inc., Chicago, IL). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Using the TNM staging, 16 patients had stage I, 42 patients had stage II, 21 patients had stage III, and 10 patients had stage IV disease.

In the control normal gastric mucosa, the anti-cyclin E and anti-p53 antibodies stained none of the glandular epithelial cells. Cyclin E expression (score 1, 2, and 3) was shown in 45 tumors (50.6%). Cyclin E overexpression (strong staining, score 2 and 3) was seen in 35 tumors (39.3%). When the 45 positive tumors were scored semiquantitatively, three tumors showed cyclin E immunoreactivity in $<5\%$ of all tumor cells (score 0), seven tumors in 5% to 25% of tumor cells (score 1), 19 tumors in 26% to 50% of tumor cells (score 2), and in 16 tumors, cyclin E expression was detected in $>50\%$ of all tumor cells (score 3). The staining was nuclear (Fig. 1A and B). Cytoplasmic staining without nuclear staining was seen in only one case and was regarded as negative. The stained cells were located mainly at the invasive fronts of the tumors, and the staining intensity was also stronger at these sites in comparisons to the other parts of the tumors. For p53, nuclear staining (Fig. 1C and D) was detected in 46 of 89 tumors studied (51.7%).

Table 1 summarizes the associations between cyclin E overexpression and various clinicopathologic variables. The incidence of cyclin E overexpression showed a significant tendency to be higher in deeply invasive cancers ($P < 0.0001$), in cancers with lymph node metastasis ($P = 0.003$), and in tumors with advanced stages ($P < 0.0001$). The incidence of cyclin E overexpression increased as the pathologic stage advanced ($P < 0.0003$). However, there were no significant associations between cyclin E overexpression and other variables. There was a significant correlation between cyclin E overexpression and the abnormal accumulation of p53 protein in tumor cells ($P < 0.0001$). Of 35 cyclin E strongly positive tumors, 29 (82.9%) showed p53 expression.

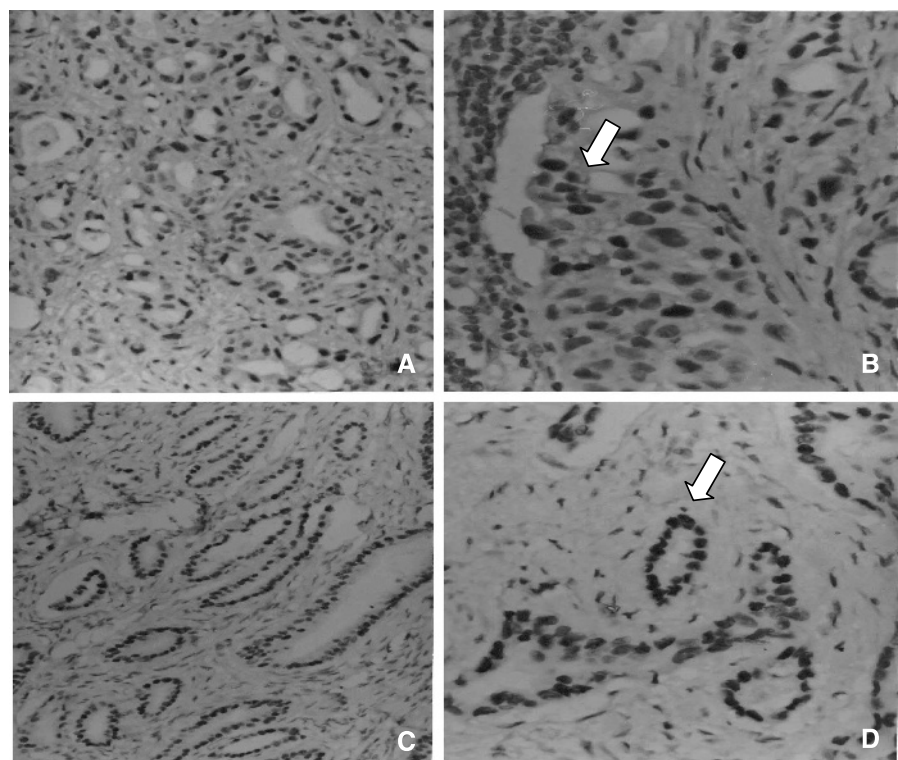


Fig. 1 A and B, immunostaining of cyclin E in moderately differentiated intestinal gastric adenocarcinoma. Nuclei at the tip of the arrow are cyclin E-positive cells. Original magnification, $\times 200$ (A), $\times 400$ (B). C and D, immunostaining of p53 in moderately differentiated intestinal gastric adenocarcinoma. Nuclei at the tip of the arrows are p53-positive cells. Original magnification, $\times 200$ (C), $\times 400$ (D).

As shown on Table 2, tumors that were strongly positive for cyclin E showed a trend towards a poorer prognosis than the other tumors ($P < 0.0001$). Similarly, tumors positive for p53 showed a trend towards a poorer prognosis than the p53 negative tumors ($P = 0.0011$). The 5-year survival of the patients with positive cyclin E tumors was 14% compared with 65% for those patients with negative tumors (Fig. 2). When cyclin E and p53 were combined, patients with both strong positivity for cyclin E and positivity for p53 had a significantly poorer prognosis than other patients ($P < 0.0001$; Fig. 3). Table 2 showed the associations between clinicopathologic characteristics and survival. Depth of invasion, lymph node metastasis, tumor advanced stage, cyclin E overexpression, and p53 expression were significantly associated with poor survival.

A Cox regression survival analysis revealed that patients with combined cyclin E overexpression and p53 expression had a significantly poorer prognosis than other patients (hazard ratio, 3.12; $P = 0.009$). After adjusting for other variables in the model (Table 3), none of the other variables in the model had a significant effect on survival time. When stepwise selection method was used, combined cyclin E overexpression and p53 expression ($P = 0.004$) and tumor stage ($P = 0.003$) were the only significant predictors of survival. On the other hand, when cyclin E and p53 were entered in the model separately, none of them had a reliable effect on survival after taking into account other possible variables.

Table 1 Clinicopathologic characteristics of 89 patients with gastric cancer according to cyclin E expression

Clinicopathologic characteristics	No. patients	Cyclin E expression		<i>P</i>
		Strongly positive	Negative or weakly positive	
Total	89	35	54	
Age (y)				
<40	17	9	8	0.201
≥40	72	26	46	
Sex				
Male	59	21	38	0.312
Female	30	14	16	
Tumor site				
Upper third, entire stomach	16	8	8	0.334
Lower third, middle third	73	27	46	
Lauren type				
Intestinal	59	20	39	0.142
Diffuse	30	15	15	
Histologic grade				
Well and moderately differentiated	43	14	29	0.206
Poorly differentiated	46	21	25	
Depth of invasion				
T1 and T2	45	7	38	<0.0001
T3 and 4	44	28	16	
Lymph node metastasis				
Presence	63	31	32	0.003
Absence	26	4	22	
TNM stage				
I, II	58	9	49	<0.0001
III, IV	31	26	5	
<i>H. pylori</i> infection				
Presence	34	12	22	0.540
Absence	55	23	32	

Table 2 Univariate analysis of factors predicting survival

Clinicopathologic characteristics	Univariate analysis		
	Hazard ratio	95% Confidence interval	<i>P</i>
Depth of invasion			
T1, T2	1		
T3, T4	5.79	2.77, 12.10	<0.0001
Lymph node metastasis			
Absence	1		
Presence	2.27	1.01, 5.07	0.0466
TNM stage			
I, II	1		
III, IV	7.07	3.47, 14.43	<0.0001
Cyclin E			
Negative or weakly positive	1		
Strongly positive	5.32	2.60, 10.87	<0.0001
P53			
Absence	1		
Presence	3.57	1.66, 7.65	0.0011
Cyclin E and P53			
Other cases*	1		
Combined cyclin E overexpression and p53 expression	5.88	2.88, 11.99	<0.0001

*Other cases are cyclin E negative and p53 negative, cyclin E negative and p53 positive, and cyclin E positive and p53 negative.

DISCUSSION

In the last decade, several studies concerning cyclin E overexpression and/or amplification in gastric cancer have been reported (28–49), and these have emanated mainly from Japan, Korea, and China. All these studies showed that cyclin E alterations are a consistent finding in gastric cancer. To our knowledge, this is the first study regarding cyclin E overexpression in gastric cancer from Jordan.

The rate of cyclin E overexpression in gastric cancer from our patients is within the range of the reported figures. The wide range in the reported rates of cyclin E expression is not totally unexpected given the complexity of the cell cycle and the entangled interrelationship between the key proteins (19).

We noticed that there was a heterogeneous intratumoral expression of cyclin E. This was also reported by other investigators (32, 33, 36, 43). Only in one of our cases, there was cytoplasmic staining. Most studies have localized the cyclin E to the nucleus of the tumor. Cytoplasmic staining, which was occasionally reported may represent a post-translational event. Accumulation of cytoplasmic cyclin E may reflect increased protein synthesis, decreased degradation, or failure of cyclin E nuclear transfer (18, 46).

We did not find significant association between cyclin E overexpression and the age or gender of the patients, nor with the site of the tumors. This lack of association with these variables was also reported by most other reports (33–35, 45, 46).

Intense cyclin E overexpression occurred in our intestinal as well as our diffuse histologic subtypes and there was no significant difference in cyclin E overexpression between the two subtypes. Although a distinct genetic pathway exists for gastric cancer of different histologic subtypes (7–11), amplification and/or overexpression of cyclin E have been reported in all histologic subtypes of gastric cancer, and the

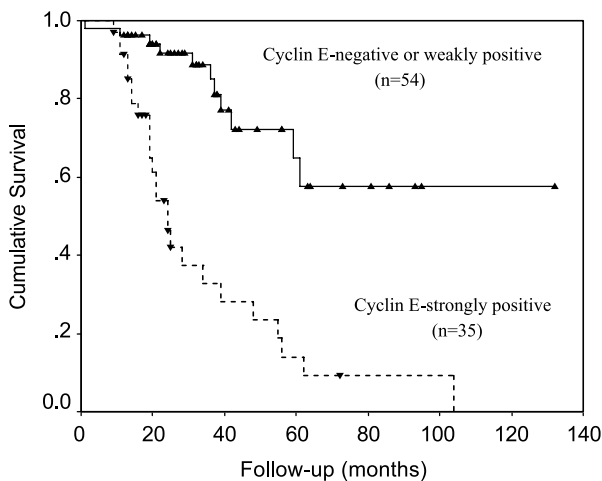


Fig. 2 The Kaplan-Meier plotting for the cumulative 5-year survival of the 89 patients with gastric adenocarcinoma, stratified according to cyclin E overexpression status. The patients with tumors strongly positive for cyclin E had significantly poorer prognosis than the other patients ($P < 0.0001$, log-rank test).

incidence of these alterations did not differ among the histologic types (34–36, 44, 46). In accordance with other reports (29, 31, 33–36), we found no significant difference in the incidence of cyclin E overexpression between well-differentiated tumors and poorly differentiated ones. However, other investigators reported a correlation between cyclin E expression and well-differentiated tumors (28, 32, 39, 43).

In agreement with other reports (19, 28, 33, 40), our comparative analysis of cyclin E overexpression and clinicopathologic variables revealed that there was a significant association between advanced cancers and cyclin E overexpression. In gastric cancer, the role of cyclin E as a prognosticator is controversial. Many studies have reported that in gastric cancer, cyclin E amplification and/or overexpression in conjunction with decreased expression of some CDKs such as p21 and p27 correlates well with the depth of penetration, poor grade, lymph node metastasis, lymphatic infiltration, advanced stages, recurrence, poorer outcome, and decrease survival. Accordingly, these studies suggested that cyclin E might be used as a prognostic indicator (28–35, 40, 45). It has been suggested that cyclin E overexpression promotes tumor cell infiltration into deeper layer and that tumors that express cyclin E have a relatively higher malignancy (32). The correlation of cyclin E expression with an adverse prognosis suggests that cyclin E promotes not only the development, but also the progression of gastric cancer by causing unbridled cellular proliferation and division (28, 29). On the other hand, there are also studies that failed to show such effect on prognosis (36–38, 46). Chetty and Sitti (46) reported that there was no significant association between cyclin E expression and any clinicopathologic features. These authors reported that 45.1% and 38.1% of gastric cancer with and without nodal metastasis, respectively, showed cyclin E overexpression. They suggested that cyclin E expression alone has no role as a prognostic indicator in gastric cancer. Despite this, some of these studies, which did not show a significant association between tumor stage and cyclin E expression, reported that cyclin E overexpression was associated

with poor survival (41, 46). Although Muller et al. did not find correlation between cyclin E expression and the traditional prognostic variables; however, cyclin E expression was associated with high tumor cell proliferation (36). Similarly, Yasui et al. (29) reported that cancers showing cyclin E overexpression were more likely than cancers without such overexpression to have a high degree of tumor cell proliferation.

In contrary to most other studies, there are only two studies (38, 39) that seem to be at odds with the general trend that cyclin E is associated with poor prognosis. Myung et al. (38) reported that advanced gastric cancer showed significantly lower scores of cyclin E expression than tubular adenomas and early gastric cancer. No explanation for the decreased expression of cyclin E during tumor progression was given. This phenomenon of decreased expression of cyclin E during tumor progression was also reported in skin and bladder cancers (25, 27). In bladder carcinoma, Kamai et al. (25) reported that low staining of cyclin E and high Ki-67 index correlated with poorly differentiated grade, muscle invasion, and lymph node metastasis. Cyclin E protein levels was inversely related with Ki-67. Different regulatory mechanisms in expression of cyclin E among different tumors may exist. It has been suggested that cellular down-regulation of cyclin E may be an attempt to offset loss of p27 expression during tumor growth via a feedback inhibitory loop (25). On the other hand, the association of high cyclin E level with good prognosis in cervical and bladder cancers may be explained by that cyclin E is functionally inactivated by high level of p21 (18, 25, 26). The other study, which presented somewhat different results is the report by Takano et al. (39) who reported that significant differences were noted with cyclin E positive versus negative for well-differentiated and less depth of cancer invasion, and that cyclin E positivity was associated with a favorable prognosis (39). They suggested that accumulation of inactive forms of cyclin E-CDK2 complexes might explain their results. These authors suggested that although cyclin E overexpression may be related to progression and poor prognosis in other tumors, gastric

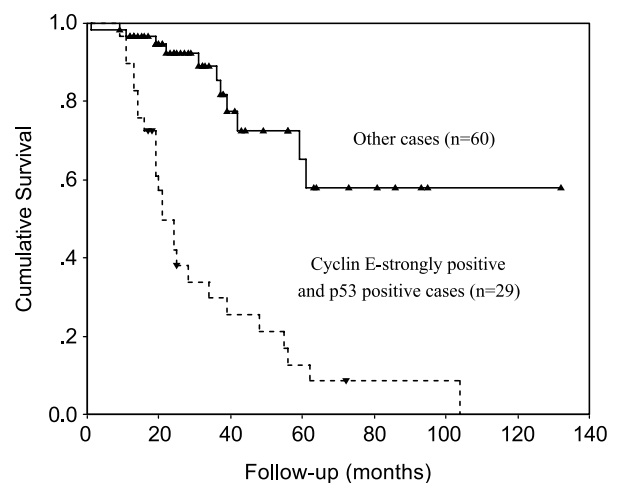


Fig. 3 The Kaplan-Meier plotting for the cumulative 5-year survival of the patients with gastric adenocarcinoma, stratified according to cyclin E and p53 expression status. Patients who had tumors with combined cyclin E overexpression and p53 expression had significantly poorer prognosis than the other patients ($P < 0.0001$, log-rank test).

Table 3 Cox regression survival analysis of factors predicting survival time of patients with gastric cancers

Variable	Hazard ratio	95% Confidence interval	P
Age, ≥ 40 versus < 40 y	2.05	0.66, 6.31	0.212
Sex, female versus male	1.21	0.41, 3.60	0.730
Histology, diffuse versus intestinal	2.53	0.89, 7.20	0.082
Lymph node metastasis, present versus absent	1.68	0.40, 7.01	0.477
<i>H. pylori</i> infection, present versus absent	0.96	0.34, 2.70	0.934
Cyclin E overexpression and P53, expression versus others	3.12	1.33, 7.30	0.009
TNM stage, III and IV versus I and II	0.66	0.11, 3.87	0.649
Depth of invasion, T3 and T4 versus T1 and T2	3.33	0.58, 19.10	0.177
Site, upper, entire versus middle and lower	1.20	0.35, 4.06	0.769
Grade, moderately to well differentiated versus poorly differentiated	0.71	0.28, 1.78	0.467

cancer are exceptional in this respect. The disparity in the results obtained by most reports including ours and the other few studies that showed no correlation with prognosis is not readily explained. Chetty and Sitti (46) had discounted technical reasons because all their negative cases were repeated and run in conjunction with a positive control case.

In this study, cyclin E overexpression was a significant prognostic factor with regard to survival. Patients with tumors exhibiting low cyclin E level were significantly less likely to die of their disease at 5 years than were patients with cancers with high levels of cyclin E. This is in agreement with other reports (33–35, 40).

It has been suggested that combined abnormalities of both cyclin E and p53, which are cell cycle regulators that act in the same pathway might increase the malignant potential of gastric cancer (33). In accordance with the Sakaguchi et al. report (33), we found that when cyclin E and p53 were combined, patients with both strong positivity for cyclin E and positivity for p53 had a significantly poorer prognosis than other patients ($P < 0.009$). Our result showed that combined cyclin E overexpression and p53 expression was independent prognostic factor with regard to survival (Table 3).

Our observations are consistent with the findings of Chetty and Sitti (46), as we did not find significant association between

cyclin E overexpression and the presence of *H. pylori* infection. Similarly, Wu et al. reported that there was no correlation between *H. pylori* infection and the alteration of five different genes in gastric cancer (9). On the other hand, Yu et al. reported that cyclin E expression was more frequent, although not significantly, in *H. pylori*-infected tissues (44).

Molecular diagnosis gives new opportunities for early cancer diagnosis and more accurate evaluation of prognosis or grade of malignancy and may provide a chance for target therapy. Assessment of cyclin E in preoperative biopsies may provide valuable information in stratifying patients with gastric cancer for different treatment protocols (18). Whether cyclin E-positive gastric cancer requires more radical therapies can be elucidated by future clinical trials. It seems that immunohistochemical detection of cyclin E is a sensitive tool for identifying subgroup of patients who may be at higher risk. But, as with esophageal adenocarcinoma, and given that multiple genetic alterations, which are implicated in the natural history of gastric cancer, a combination of carefully validated biomarkers including cyclin E, p53, and CDKIs might improve still further the predictive value of the molecular approach (52, 53). There is accumulating evidence that cyclin E, p27, and p53 are useful adjuncts to traditional prognostication indices (19, 33).

In conclusions we have found that cyclin E protein expression is deregulated in gastric cancer, and its overexpression is a frequent finding in gastric cancer. The combined evaluation of cyclin E and p53 expressions in gastric cancer tissues aids in predicting the clinical prognosis for surgically treated patients with gastric cancer. This coexpression might be an independent prognosticator for survival in patients with gastric cancer.

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