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Original article

Role of cyclin E and *p53* expression in progression of early gastric cancer

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Abstract:
Background. **To elucidate the role that cyclin E overexpression plays in the progression of early gastric cancer, we examined the expression of cyclin E and *p53*, as abnormal *p53* expression is linked with cyclin E overexpression in exerting adverse affects on the cell cycle.**
Methods. **Specimens from 108 early gastric cancers were stained by an immunohistochemical method, using anti-cyclin E and anti-*p53* antibodies.**
Results. **The positivity rate of cyclin E expression in early gastric cancer was 33% (36/108). Cyclin E-positive tumors invaded more deeply ($P < 0.05$), infiltrated lymphatic vessels more frequently ($P < 0.01$), showed a higher incidence of differentiated cancer ($P < 0.01$), and more often expressed *p53* ($P < 0.01$) than cyclin E-negative tumors. Differentiated cancers showing coexpression of cyclin E and *p53* were more likely to metastasize to the lymph nodes.**
Conclusions. **Overexpression of cyclin E may promote the progression of early gastric cancer.**

Key words: cyclin E, *p53*, early gastric cancer, tumor progression, immunohistochemistry

Introduction

Cyclin E, cyclin-dependent kinase 2 (CDK2), and CDK inhibitors positively and negatively regulate the G_1/S transition in the late G_1 phase of the cell cycle [1,2]. Cyclin E, a nuclear protein, acts as a G_1 cyclin in human cells. It directly promotes the G_1/S transition by combining with and activating CDK2 [1,2]. Wild-type *p53* indirectly prevents this transition, by activating the transcription of p21, a CDK inhibitor, which, in turn, inhibits the function of cyclin E-CDK2 complex [3,4]. Therefore, abnormalities of cyclin E and *p53* expression, as well as the subsequent deregulation of the G_1/S transition in the cell cycle, may result in unbridled cell proliferation and participate in the genesis and progression of various carcinomas [5].

In breast cancer, the overexpression of cyclin E at the gene, mRNA, and protein levels promotes tumorigenesis [6,7]. The correlation of cyclin E expression with an adverse prognosis [8] suggests that cyclin E promotes the progression of breast cancer. In gastric cancer, the overexpression of cyclin E at the gene and protein levels [9], as well as the high incidence of cyclin E expression in adenocarcinoma [5], suggests a role for cyclin E in carcinogenesis. Because of the adverse effects of abnormal cyclin E and *p53* expression in the cell cycle [9–11], the overexpression of cyclin E, as well as the simultaneous abnormal expression of cyclin E and *p53*, is likely to promote the progression of early gastric cancer. However, to date, no previous studies have examined this possibility in early gastric cancer. To elucidate the role of cyclin E overexpression in the progression of early gastric cancer, we examined cyclin E and *p53* expression in such cancers.

Patients and methods

Patients and specimens

We examined 108 specimens from patients with early gastric cancer (74 men and 29 women, aged 31–85 years [average, 63 years]) who had undergone gastrectomy between 1985 and 1995 at Kagoshima University Hospital. Pathological diagnosis of each tumor was done according to the *General rules for the gastric cancer study in surgery and pathology* of the Japanese Research Society for Gastric Cancer [12]. Macroscopically the

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cancers were divided into three subtypes: elevated (which included types I, IIa, and IIb), depressed (which included types IIc, III, and III + IIc), and mixed (which included types IIa + IIc and IIb + IIc). Histologically the cancers were divided into two subtypes: differentiated (which included papillary and tubular adenocarcinoma) and undifferentiated (which included signet ring cell carcinoma, mucinous, and poorly differentiated adenocarcinoma). The resected specimens were fixed in formalin and embedded in paraffin. One representative section from each patient, including both central and peripheral areas, was stained with H&E for histological evaluation. Two other consecutive sections were stained with anti-cyclin E and anti-*p53* antibodies. For control group, we used normal gastric mucosa away from tumor.

Immunohistochemical staining

Sections 3-μm-thick were stained using the avidin-biotin-peroxidase technique (ABC method), in accordance with the manufacturer's instructions for the Histofine Immunohistochemical System (Histofine; Nichirei, Tokyo, Japan) and as previously described [13]. In brief, deparaffinized tissue sections were immersed in methanol containing 0.3% H_2O_2 for 30 min to block endogenous peroxidase activity. They were then heated in citrate buffer (0.01 M, pH 6.5) to retrieve antigenicity (for cyclin E, at 100 °C, for 30 min; for *p53*, at 120 °C, for 10 min). The sections were then incubated with anti-cyclin E antibody (diluted 1:600; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-*p53* antibody (diluted 1:500; Transduction Laboratories, Lexington, KY, USA) for at least 12 h at 4 °C, followed by incubation with biotinylated rabbit anti-mouse serum for 20 min and incubation with streptavidin-peroxidase complex for 15 min. Staining was developed by incubating the sections in diaminobenzidine tetrahydrochloride (DAB) for 5 min. The sections were then counterstained in hematoxylin, dehydrated, and mounted.

Immunohistochemical evaluation

The positively stained tumor cells were heterogeneously distributed in tumors, so 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 1000 cells was counted for each cancer and the average percentage of stained cells was calculated. Tumor cyclin E and *p53* expression was regarded as negative (−) when the proportion of stained cells was <5%, and positive (+) when the proportion of stained cells was >5% [13,14].

Statistical analysis

We assessed the clinicopathological features of the patients in relation to the expression of cyclin E and *p53* using Student's *t*-test and the χ^2 test. For all statistical analyses, StatView Version 4.5 software (Abacus Concepts, Berkeley, CA, USA) was used, with statistical significance defined as $P < 0.05$.

Results

Expression of cyclin E in control and cancerous tissue

In control gastric mucosae, none of the glandular epithelial cells were stained by the cyclin E antibody. The cytoplasm of endothelial cells in vessels was positively stained; however, the nuclei were negative. The cytoplasm of some lymphocytes was slightly stained, while the nuclei were also negative. In gastric cancers (Fig. 1), cyclin E staining was localized to the nuclei, and stained cells were heterogeneously distributed. The percentage of cells positive for cyclin E or *p53* expression in different tumors ranged from 0 to 81.5% and 0 to 75%, respectively. The positive cells were located mainly at the invasive fronts of cancer, and were gathered focally; there were few positive cells in the central parts of tumors. The staining intensity was stronger in the invasive fronts of tumors than in the other parts.

Correlation of cyclin E expression with clinicopathological features

The positivity rate for cyclin E expression in the early gastric cancers was 33% (36/108) (Table 1). There was a higher rate of positive cyclin E expression in tumors from men than from women. Tumors positive for cyclin

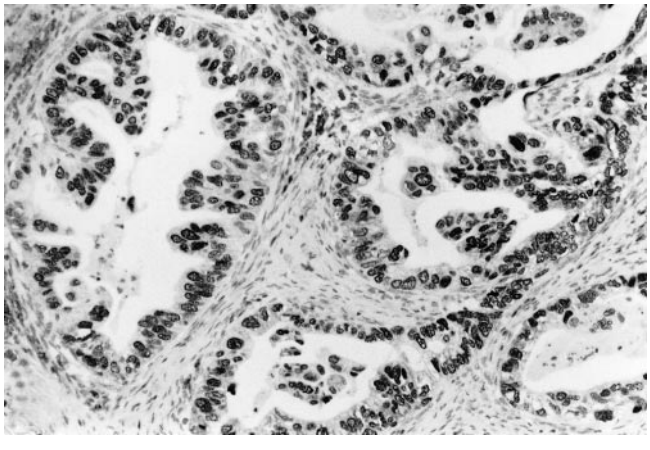

Fig. 1. Expression of cyclin E in cancerous tissue: immunoreactivity was localized in the nuclei of stained cells. Immunohistochemistry ×200

E expression were generally smaller than cyclin E-negative tumors. Tumors in the antrum were more likely to express cyclin E than tumors in other parts of the stomach. Differentiated early gastric cancers expressed cyclin E significantly more frequently than undifferentiated tumors. Tumors invading the submucosal layer had a higher positive rate than those showing mucosal invasion ($P < 0.05$), and tumors infiltrating the lymphatic vessels had a higher positive rate than tumors not showing lymphatic infiltration ($P < 0.01$). Tumors with venous invasion expressed cyclin E slightly more frequently than tumors without venous invasion, but the difference was not significant. Cyclin E expression was not correlated with other clinicopathological features.

Correlation of p53 *expression with clinicopathological features*

The positivity rate for *p53* expression was 32% (35/108) (Table 1) (Fig. 2). There was a higher rate of positive *p53* expression in tumors from men than from women. The *p53*-positive patients were older than the *p53*-negative group ($P < 0.01$). Differentiated early gastric cancers expressed *p53* significantly more frequently than undifferentiated tumors. Carcinomas invading the submucosa had a higher positive rate than those invading the mucasa, but the difference was not significant. Tumors invading the lymphatic vessels had a higher positive rate than those not showing lymphatic invasion, but this difference was also not significant. *p53* expression was not correlated with tumor size, location, or gross type, or with lymph node metastases.

Correlation of cyclin E expression with p53 *expression*

Cyclin E expression resembled *p53* expression in that their positivity rates were roughly equal and expression was more frequent in men, in differentiated tumors, in tumors with submucosa, invasion, and in tumors with venous invasion. Neither cyclin E, *p53* expression nor was correlated with gross type or with lymph node metastasis. As shown in Table 2, cyclin E expression was significantly correlated with *p53* expression. Of the

Table 1. Correlation of patients' clinicopathological features with cyclin E and *p53* expression in early gastric cancer

		Cyclin E			*p53*		
Features	Number of patients	(+) (%)	(−)	*P* value	(+) (%)	(−)	*P* value
Total	108	36 (33)	72		35 (32)	73	
Sex							
F	29	5 (17)	24	0.032	4 (14)	25	0.012
M	79	31 (39)	48		31 (39)	48	
Age (years)		66 ± 10	61 ± 13	NS	68 ± 10	61 ± 14	0.006
Size of tumor (mm)		22 ± 11	31 ± 19	0.012	24 ± 15	30 ± 18	NS
Location							
C	27	6 (22)	21	0.01	9 (33)	18	NS
M	31	6 (19)	25		6 (19)	25	
A	50	24 (48)	26		20 (40)	30	
Gross type							
Ele.	19	6 (32)	13	NS	7 (37)	12	NS
Dep.	76	24 (32)	52		21 (28)	55	
Mixed	13	6 (46)	7		7 (54)	6	
Histology							
Dif.	78	31 (40)	47	0.022	33 (42)	45	<0.001
Undif.	30	5 (17)	25		2 (7)	28	
Depth of invasion							
Mucosa	57	14 (25)	43	0.04	14 (25)	43	0.06
Submucosa	51	22 (43)	29		21 (41)	30	
Venous invasion							
(−)	90	27 (30)	63	NS	26 (28)	55	NS
(+)	18	9 (50)	9		9 (50)	9	
Lymphatic invasion							
(−)	76	19 (25)	57	0.01	21 (28)	55	NS
(+)	32	17 (53)	15		14 (44)	18	
Lymph node metastasis							
(−)	95	31 (33)	64	NS	29 (31)	66	NS
(+)	13	5 (38)	8		6 (46)	7	

C, Upper third of stomach; M, middle third of stomach; *A*, lower third of stomach; Ele, elevated; Dep, depressed; Mixed, mixed

cyclin E-positive tumors, 53.8% (19/36) expressed *p53*, while only 22.2% (16/72) of the cyclin E-negative tumors expressed *p53*.

Fig. 2. *p53* overexpression in gastric cancer cells; the nuclei were stained. Immunohistochemistry, ×200

Table 2. Correlation of cyclin E and *p53* expression in early gastric cancers

		Expression of p53	
		−	+
Expression of cyclin E	−	56 (77.8%)	16 (22.2%)
	+	17 (47.2%)*	19 (53.8%)*

* $P < 0.01$

Correlation of p53 *and cyclin E coexpression with clinicopathological features*

Differentiated early gastric cancers had a significantly higher coexpression rate of cyclin E and *p53* than undifferentiated tumors (Table 3). Differentiated tumors with lymphatic vessel invasion more frequently expressed both cyclin E and *p53* than those without invasion ($P < 0.01$) (Table 4). Differentiated tumors with lymph node metastases more frequently expressed cyclin E and *p53* synchronously than did those without metastasis with the difference almost reaching significance ($P = 0.058$). The coexpression of cyclin E and *p53* in early differentiated gastric cancers was not related to any other clinicopathological features of those cancers.

Discussion

Our finding that control gastric mucosa was negative for cyclin E and that cyclin E expression was positive in 33% of the early gastric cancers suggests not only that the level of cyclin E expression is lower under normal conditions but also that the overexpression of cyclin E participates in the genesis of early gastric cancer. Tumors invading the submucosa more frequently expressed cyclin E, than tumors not invading this layer confirming previous results in a small series [5]. As cyclin E expression is a marker of tumor proliferation [15], these results suggest that cancer cell proliferation is accelerated when tumors invade the submucosa. This conclusion is further supported by results of studies of proliferating cell nuclear antigen [16] and cyclin E gene

Table 3. Co-expression of cyclin E and *p53* in early gastric cancers: Correlation with histological type

		Co-expression of cyclin E and *p53*				
Histological type	Number of patients	*p53* − Cyclin E −	*p53* + Cyclin E −	*p53* − Cyclin E +	*p53* + Cyclin E +	*P* value
Dif.	78	33 (42%)	14 (18%)	12 (15%)	19 (25%)	<0.01
Undif.	30	23 (77%)	2 (6%)	5 (17%)	0 (0%)	

Table 4. Co-expression of cyclin E and *p53* in early well differentiated gastric cancers ($n = 78$): Correlation with lymphatic invasion

			Co-expression of cyclin E and *p53*				
Features		Number of patients	*p53* − Cyclin E −	*p53* + Cyclin E −	*p53* − Cyclin E +	*p53* + Cyclin E +	*P* value
Lymphatic vessel invasion	−	53	25 (47%)	12 (23%)	9 (17%)	7 (13%)	<0.01
	+	25	8 (32%)	2 (8%)	3 (12%)	12 (48%)	
Lymph node metastases	−	70	31 (44%)	13 (19%)	12 (17%)	14 (20%)	0.058
	+	8	2 (25%)	1 (12%)	0 (0%)	5 (63%)	

amplification [9], which also suggest that cyclin E overexpression promotes tumor cell infiltration into deeper layers. Our finding that cyclin E-positive cells were aggregated at the invasive fronts of tumors indicates that the cancer cells in this part proliferate more quickly than in the other parts of the tumor.

We found that differentiated tumors in early gastric cancer expressed cyclin E more frequently than undifferentiated tumors, suggesting that the proliferative mechanisms of the two types of tumor differ and that abnormal cyclin E expression plays a role in the progression of early differentiated gastric cancer. We also found that early gastric cancers with cyclin E expression were significantly more likely to infiltrate lymphatic vessels. It has been shown that gastric cancer cells which overexpress cyclin E proliferate more quickly [5]. As the overexpression of cyclin E in tumor cells may result in cyclin E bypassing related cyclin-CDK feedback loops, providing yet another mechanism by which tumors can gain a growth advantage [17], our findings seem reasonable and suggest that early gastric cancers which express cyclin E have a relatively higher malignancy.

Our *p53* positivity rate of 32% was identical to the findings of Fonseca et al. [18] in early gastric cancer. Our findings that differentiated carcinomas which invaded the submucosa or lymphatic vessels had a higher *p53* positivity rate than those that did not display these characteristics are identical to the findings of Joypaul et al. [19], and confirm that abnormal *p53* expression has adverse effects in early gastric cancer [18].

Cyclin E expression resembled that of *p53*, and cyclin E-positive tumors expressed *p53* more frequently than cyclin E-negative tumors, suggestive of a correlation between cyclin E and *p53* expression. As the coexpression of cyclin E and *p53* correlated with histological type and was more frequent in differentiated tumors, we examined the correlation between coexpression and pathological features in differentiated tumors. Differentiated tumors which coexpressed cyclin E and *p53* invaded lymphatic vessels more frequently and had a greater tendency to metastasize to the lymph nodes. The *p53* protein stained in our gastric cancers is most probably the mutated-type *p53* [20], which has lost the function (to activate *p21*) of wild type *p53*. Thus the G1/S transition is accelerated in tumor cells expressing mutated type *p53*. Overexpression of cyclin E also promotes cell proliferation. Therefore, tumors with cyclin E and *p53* coexpression may proliferate more quickly than those without this feature. Thus, the synchronous overexpression of cyclin E and *p53* may promote tumor cell invasion of the lymphatic system. These results suggest that differentiated early gastric cancer showing coexpression of cyclin E and *p53* is more likely to progress to an advanced stage. Accordingly patients with such coexpression should receive aggressive adjuvant treatment and be closely monitored during follow-up.

In summary, the higher frequencies of deep invasion, lymphatic vessel invasion, venous invasion, *p53* expression, and differentiated cancer in our cyclin E-positive patients with early gastric cancer suggest that overexpression of cyclin E promotes the progression of early gastric cancer. The higher frequency of lymphatic invasion indicates that differentiated early gastric cancer coexpressing cyclin E and *p53* is more likely to progress to an advanced stage.

References

1. Sherr CJ. G_1 phase progression: Cyclin on cue. Cell 1994;79:551–5.
2. Hunter T, Pines J. Cyclins and cancer II: Cyclin E and CDK inhibitors come of age. Cell 1994;79:573–82.
3. el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, et al. WAF1, potential mediator of p53 tumor suppression. Cell 1993;75:817–25.
4. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. The p21 Cdk-interacting protein Cip 1 is a potent inhibitor of G_1 cyclin-dependent kinases. Cell 1993;75:805–16.
5. Yasui W, Akama Y, Kuniyasu H, Yokozaki S, Semba S, Shimamoto F, et al. Expression of cyclin E in human gastric adenomas and adenocarcinomas: Correlation with proliferative activity and p53 status. J Exp Ther Oncol 1996;1:88–94.
6. Keyomarsi K, Pardee AB. Redundant cyclin overexpression and gene amplification in breast cancer cells. Proc Natl Acad Sci USA 1993;90:1112–6.
7. Gray-Bablin J, Zalvide J, Fox MP, Knickerbocker CJ, Decaprio JA, Keyomarsi K. Cyclin E, a redundant cyclin in breast cancer. Proc Natl Acad Sci USA 1996;93:15 215–20.
8. Porter PL, Malone KE, Heagerty PJ, Alexander GM, Gatti LA, Firpo EJ, et al. Expression of cell-cycle regulators $p27^{Kip1}$ and cyclin E, alone and in combination, correlate with survival in young breast cancer patients. Nature Medicine 1997;3:222–5.
9. Akama Y, Yasui W, Yokozaki H, Kuniyasu H, Kitahara K, Ishikawa T, et al. Frequent amplification of the cyclin E gene in human gastric carcinomas. Jpn J Cancer Res 1995;86:617–21.
10. Vogelstein B, Kinzler KW. p53 function and dysfunction. Cell 1992;70:523–6.
11. Levine AJ, Momand J, Finlay GA. The p53 tumour suppressor gene. Nature 1991;351:453–6.
12. Japanese Research Society for Gastric Cancer. The general rules for the gastric cancer study in surgery and pathology. Jpn J Surg 1981;11:127–39.
13. Yasui W, Kuniyasu H, Yokozaki H, Semba S, Shimamoto F, Tahara E. Expression of cyclin E in colorectal adenomas and adenocarcinomas: Correlation with expression of Ki-67 antigen and p53 protein. Virchows Arch 1996;429:13–9.
14. Tang HH, Hokita S, Che XM, Baba M, Aridome K, Kijima F, Tanabe G, et al. Comparison of p53 expression in proximal and distal gastric cancer: Histopathologic correlation and prognostic significance. Ann Surg Oncol 1997;4:470–4.
15. Dutta A, Chandra R, Leiter LM, Lester S. Cyclins as markers of tumor proliferation: Immunocytochemical studies in breast cancer. Proc Natl Acad Sci USA 1995;92:5386–90.
16. Iida A, Hirose K, Arai M, Yamaguchi A, Nakagawara G. Relationships among the expression of epidermal growth factor receptor, proliferating cell nuclear antigen labeling index, and lymph node metastasis in gastric cancer. Oncology 1995;52:189–95.

17. Gong J, Traganos F, Darzynkiewicz Z. Threshold expression of cyclin E but not D type cyclins characterizes normal and tumour cells entering S phase. Cell Prolif 1995;28:337–46.
18. Fonseca L, Yonemura Y, De-Aretxabala X, Yamaguchi A, Miwa K, Miyazaki I. p53 detection as a prognostic factor in early gastric cancer. Oncology 1994; 51:485–90.
19. Joypaul BV, Hopwood D, Newman EL, Qureshi S, Grant A, Ogston SA, et al. The prognostic significance of the accumulation of p53 tumour suppressor gene protein in gastric adenocarcinoma. Br J Cancer 1994; 69:943–6.
20. Ayhan A, Yasui W, Yokozaki H. Genetic abnormalities and expression of p53 in human colon carcinomas. Int J Oncol 1992;1:431–7.