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ORIGINAL PAPER

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Overexpression of cyclin B1 in gastric cancer and its clinicopathological significance: an immunohistological study

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Abstract *Purpose*: Cyclin B1 is a key regulator of progression through the G2/M transition during the cell cycle. Although cyclin B1 proteins are overexpressed in various types of human cancers, the relationship between cyclin B1 status in gastric cancer and its clinical significance remains unknown. *Methods*: We examined cyclin B1 expression by immunohistological means in 61 patients with gastric cancer in terms of histological type, tumor invasion, and metastatic behavior. Specimens were considered positive when the cytoplasm of over 10% of the cancer cell population was stained. *Results*: Cyclin B1 was overexpressed in 32 (53%) of 61 patients with gastric cancer. Tumors that expressed cyclin B1 were predominant in older patients, in well- and moderately differentiated adenocarcinomas and in expanding-growth type tumors. Conversely, expression of cyclin B1 was lower in poorly differentiated adenocarcinomas, and in those of the infiltrative growth type. Moreover, the disease was more advanced (stages III and IV) and widespread nodal involvement was more frequent when cyclin B1 expression was low. Logistic regression analyses showed that histological type is a significant factor related to cyclin B1 protein expression. *Conclusions*: These findings suggested that cyclin B1 protein overexpression is closely associated with less aggressive tumor behavior. Therefore, G2/M cyclin alternatives and the possible role of cyclins in cancer development warrants further attention.

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Introduction

Cyclins play an important role in regulating checkpoints in the cell cycle. Progression thorough the cell cycle is governed by a family of cyclin-dependent kinases (cdks) that are activated by binding to cyclin proteins (Sherr 1996). Passage through the G1 and S phases is regulated by the activities of cyclin D-, cyclin E-, and cyclin A-associated kinases. Cyclin B1 activates cdc2, which regulates cell progression through the G2 and M phases (Pines and Hunter 1990). Cyclin B1 arises in the cytoplasm of cells in S-phase, and then it is transported to the nucleus at the G2/M transition where it is broken down during anaphase via an ubiquitin-dependent pathway (Holloway et al. 1993).

Dysregulated expression of these cyclins, cdks, or both may lead to uncontrolled cell growth and malignant transformation. Indeed, uncontrolled cell division is one of the key features of tumor cells. Cyclin D1 is overexpressed and/or amplified in a variety of human cancers, including those of the oesophagus, head and neck, lung, liver and breast (Sherr 1996). Cyclin D1 overexpression and/or amplification is considered to be of prognostic importance in patients with most of these types of tumors. Cyclin B1 is overexpressed in breast (Dutta et al. 1995; Kawamoto et al. 1997), colon (Wang et al. 1997), prostate (Mashal et al. 1996), oral (Kushner et al. 1999), esophageal (Murakami et al. 1999), and lung carcinomas (Soria et al. 2000). Its prognostic value has been suggested in patients with squamous cell carcinoma of the esophagus (Murakami et al. 1999). However, only a few investigators have found cyclin B1 overexpression in gastric cancer. The present study examines whether or not the overexpression of G2/M cyclin is related to the development and metastatic potential of tumors.

Materials and methods

Patients and clinicopathology

We examined 61 samples of primary gastric cancer resected between 1983 and 1995. The specimens were fixed in 10% formalin and embedded in paraffin. The type of cancer was histopathologically diagnosed by reviewing hematoxylin and eosin-stained sections. Tumors were clinically or histopathologically classified according to the Japanese Classification of Gastric Carcinoma (Japanese Gastric Cancer Association 1999). After discharge from our hospital, the patients were followed up for a mean period of 25.8 months.

Immunohistochemistry for cyclin B1

Archival formalin-fixed paraffin-embedded sections (4 μm thick) were placed on silane-coated slides for immunohistochemistry. Cyclin B1 protein expression was assessed using a mouse anti-human cyclin B1 monoclonal antibody (Transduction Laboratories, Lexington, Ky., USA). Sections were autoclaved in 10 mM citrate buffer (heated at 121 °C) for 10 min. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min, then the sections were incubated with normal goat serum for 30 min followed by cyclin B1 antibody at room temperature for 2 h. Sections were immunostained by the two-step method using EnVision+ (K4000: DAKO; Calif., USA) and counterstained with hematoxylin before mounting.

Cyclin B1 protein appears in the cytoplasm of cells at the S- and G2-phases and is transported to the nucleus at the G2/M transition. The degree of cyclin B1 staining was determined in the present study as the percentage of cells exhibiting cyclin B1 cytoplasmic staining. Expression was considered positive if the cytoplasm of over 10% of the cell population was stained.

Statistical analyses

Statistical association between cyclin B1 expression and various clinicopathological factors was determined using the Chi-square test for categorical variables. Independent factors influencing cyclin B1 expression were then determined by logistic regression analysis. The cumulative survival rates were calculated using Kaplan-Meier methods and the statistical significance of differences was determined using the log-rank test.

Results

Immunohistochemical expression of cyclin B1 in gastric cancer and normal mucosal tissues

Among the 61 tumors examined, 32 (52.5%) positively immunoreacted with cyclin B1 antibody. Cyclin B1 overexpression was dominant in the cytoplasm of cancer cells (Fig. 1). Cyclin B1 was focally immunoexpressed in the cytoplasm or nucleus in nine of 46 normal gastric mucosa samples (19.6%). Therefore, cyclin B1 was more frequently expressed in tumor tissues than in normal mucosa.

Correlation between cyclin B1 overexpression and clinicopathological factors

Table 1 shows the relationship between cyclin B1 expression and clinicopathological factors in patients with gastric cancer. Cyclin B1 expression was not significantly associated with clinicopathological parameters, including sex, tumor location, peritoneal dissemination, liver metastasis, depth of invasion or venous invasion. The mean age of cyclin B1 positive patients was 65.3 years, which was significantly higher than the 58.8 years of cyclin B1 negative patients ($P = 0.04$). Cyclin B1 was overexpressed in 50.0% of early gastric cancers. Cyclin B1 was more frequently overexpressed in advanced cancers of the expansive growth type than the infiltrative growth type ($P = 0.02$). Twenty of 29 (69.0%) differentiated adenocarcinomas expressed high levels of cyclin B1, as opposed to only 11 of 31 (35.5%) poorly differentiated adenocarcinomas ($P = 0.005$). Less cyclin B1 was expressed in tumors with widespread nodal metastasis (pN2–4)($P = 0.03$) and in those with much lymphatic

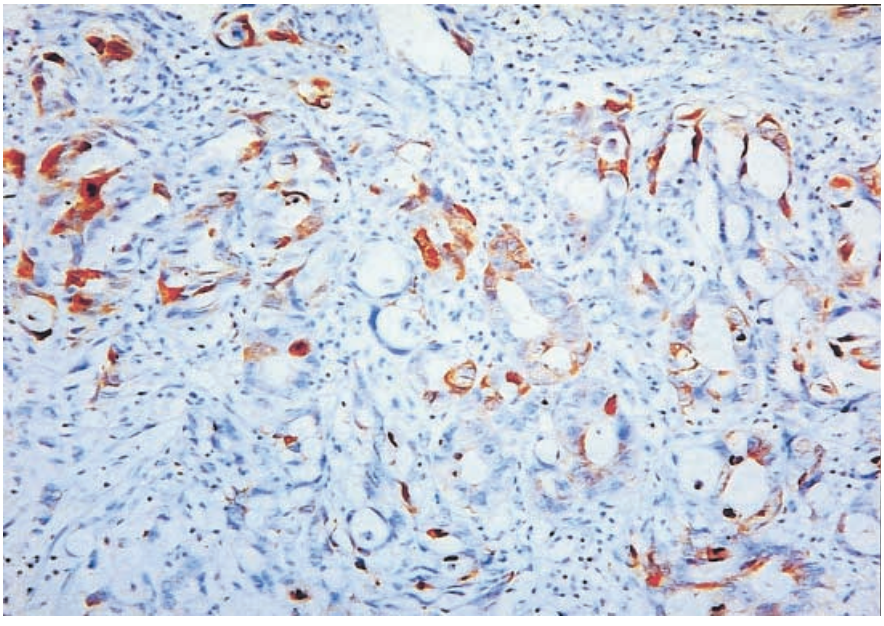

Fig. 1. Cyclin B1 staining of human gastric carcinoma (×200). Positive staining was heterogeneous in cytoplasm and nucleus of cancer cells

Table 1. Relationship between cyclin B1 expression and clinicopathological parameters

Age (years)	Cyclin B1 expression in cytoplasm		*P* value
	Negative ($n=29$)	Positive ($n=32$)	
Mean	58.8	65.3	$P=0.04$
SD	10.7	13.7	
Sex			
Male	18	20	N.S.
Female	11	12	
Location			
Upper	8	5	N.S.
Middle	9	13	
Lower	12	14	
Gross type			
B0	11	11	$P=0.02$
B1, 2	2	11	
B3, 4	16	10	
Differentiation			
Poorly	20	11	$P=0.005$
Moderately	7	7	
Well-	2	13	
Peritoneal dissemination			
Negative	24	31	N.S.
Positive	5	1	
Liver metastasis			
Negative	29	29	N.S.
Positive	0	3	
Depth of invasion			
m, sm	7	12	N.S.
mp, ss	3	7	
se, si	19	13	
Nodal metastasis			
N0	12	18	$P=0.03$
N1	2	7	
N2, 3, 4	15	7	
Stage			
ST1, 2	8	18	$P=0.02$
ST3, 4	21	14	
Lymphatic invasion			
ly0	12	21	$P=0.04$
ly1	4	6	
ly2, 3	13	5	
Venous invasion			
v0	20	24	N.S.
v1	5	1	
v2, 3	2	6	

invasion (ly2, 3) ($P=0.04$) compared with less aggressive tumors. Cyclin B1 was overexpressed in 18 of 26 (69.2%) stage I-II tumors, which was significantly higher than that of stage III-IV tumors ($P=0.02$).

Significant risk factors related to cyclin B1 overexpression were determined by logistic regression analysis. Histological type proved to significantly influence the expression of cyclin B protein (regression coefficient 3.305, relative risk 27.26, $P<0.05$).

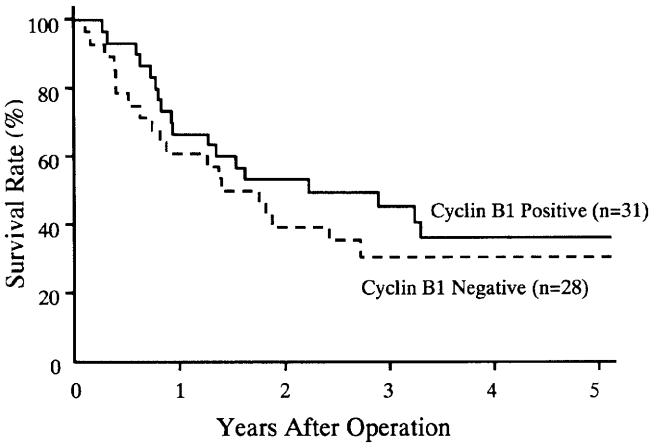

Fig. 2. Survival curves of patients with gastric cancer according to cyclin B1 positivity. Although prognosis tended to be better in patients with cyclin B1-positive tumors than with negative tumors, the difference was not significant

Cyclin B1 overexpression and prognosis (Fig. 2)

Survival curves of patients with cyclin B1 positive and negative tumors are shown in Fig. 2. Although the prognosis of patients tended to be better if they had cyclin B1 positive than negative tumors, these two groups did not significantly differ.

Discussion

Among the various cyclins, cyclin D1 and cyclin E that regulate the G1/S transition have been studied in detail. Many reports have described the overexpression and/or amplification of cyclin D1 in tumors of various organs, including those of the head and neck (Patel et al. 1997), esophagus (Roncalli et al. 1998), lung (Volm et al. 1998), liver (Nishida et al. 1994), and breast (Jares et al. 1997). Cyclin D1 correlates with the malignant behavior of these cancers and prognosis. On the other hand, Molendini et al. reported that a loss of cyclin D1 immunoexpression is a prognostic marker of human osteosarcoma (Molendini et al. 1998). Cyclin E expression is associated with malignant behaviors of gastric (Sakaguchi et al. 1998), but not of colorectal carcinoma (Yasui et al. 1996). Thus, the cyclins involved in carcinogenesis or progression may have organ specificity.

In several investigations, it has been shown that the overexpression of cyclin B1 in cancer cells associated with tumor progression or poor prognosis may be specific for either individual organs or types of histology. Cyclin B1 protein overexpression in esophageal squamous cell carcinomas statistically increases together with the depth of invasion and presence of venous invasion (Murakami et al. 1999). They also demonstrated using multivariate analyses that cyclin-B1 positivity is an independent prognostic factor. In contrast to these findings, Soria et al. considered that the overexpression of

cyclin B1 might be a prognostic marker for patients with squamous cell carcinoma of the lung but not for those with other histological types of carcinomas including adenocarcinoma (Soria et al. 2000). In adenocarcinomas of the stomach or the colorectum, cyclin B1 expression was not correlated with prognosis (Brien et al. 1998; Korenaga et al. 2002). No prognostic significance of patients with gastric cancer was obtained in our study that was in close agreement with their results. Thus, the relationship between cyclin B1 expressions and its potential prognostic value may be relevant to squamous cell carcinoma but not to adenocarcinoma.

Second, we investigated the correlation of cyclin B1 expression with the clinicopathological parameters in detail. Cyclin B1 overexpression was associated with less aggressive behavior of the tumors, including metastasis and stage of disease. Similar findings were reported by Korenaga et al. – in patients with colorectal adenocarcinoma – who uncovered a close correlation between the cyclin B1 overexpression in colorectal cancer and less strong metastatic potentiality characterized by lymph node metastases, lymphatic permeation, venous invasion and Duke's tumor stage (Korenaga et al. 2002). There is general agreement that undifferentiated gastric carcinoma, which generally corresponded to diffuse type carcinoma, was more common in patients with advanced cancer associated with infiltrative growth type and the occurrence of peritoneal dissemination (Ikeda et al. 1994; Moriguchi et al. 1991). In this study, we obtained evidence that cyclin B1 overexpression was more frequently seen in differentiated adenocarcinomas than in poorly differentiated adenocarcinomas, and the histological type of tumor proved to be a significant factor related to cyclin B1 overexpression as determined by multivariate analysis. Considering these observations, our data suggest that cyclin B1 overexpression is related to tumorigenesis but not to the progression of gastric cancer, which may be one of the pathognomonic characteristics of adenocarcinoma.

Cyclin B1 may form complexes with cdc2, of which an elevated level would be a trigger for passage through the cell cycle checkpoint, resulting in uncontrolled cell division and probable carcinogenesis or tumor progression. Although elevated levels in the complex play a critical role in G2-M transition, the nucleocytoplasmic distribution of the complex is likely to be more important for cell cycle control. In the nucleus, the kinase activity of cdc2 is controlled during the cell cycle by phosphorylation or dephosphorylation at inhibitory phosphorylation sites (Li et al. 1997). p53 regulates G2-M transition through down-regulation of cyclin B1 (Innocente et al. 1999; Park et al. 2000). On the other hand, the cytoplasmic accumulation of cyclin B1 during S and G2 phase is directed by a nuclear exporting signal (NES)-dependent transport mechanism, which appears to be important in preventing inappropriate mitosis in the presence of damaged DNA (Jin et al. 1998; Toyoshima et al. 1998). Recent studies suggest that 14–3-3s anchors cdc2 and cyclin B in the cytoplasm where the enzyme cannot be activated and mitosis is also inhibited (Taylor and Stark 2001). In this respect, the accumulation of cyclin B1 protein in the cytoplasm of gastric cancer cells could be interpreted simply as an effect of mitotic arrest on the G2-M checkpoint without the activated cyclin B1-cdc2 complex, which may not lead to uncontrolled cell division and tumor progression. This may be, in part, the reason why overexpressed cyclin B1 protein in the cytoplasm of the gastric cancer cells was closely associated with less aggressive tumor behavior.

In conclusion, the present study shows that the overexpression of cyclin B1 protein in gastric cancer is closely associated with less aggressive tumor behavior. Cyclin B1 may relate to tumorigenesis but not to the progression of adenocarcinoma of the stomach. Alternatives to G2/M cyclin and its possible role in cancer development warrants further attention.

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References

Brien TP, Depowski PL, Sheehan CE, Ross JS, McKenna BJ (1998) Prognostic factors in gastric cancer. Modern Pathol 11:870–877

Dutta A, Chandra R, Leiter LM, Lester S (1995) Cyclins as markers of tumor proliferation: immunocytochemical studies in breast cancer. Proc Natl Acad Sci USA 92:5386–5390

Holloway SL, Glotzer M, King RW, Murray AW (1993) Anaphase is initiated by proteolysis rather than by the inactivation of maturation-promoting factor. Cell 73:1393–1402

Ikeda Y, Mori M, Kamakura T, Haraguchi Y, Saku M, Sugimachi K (1994) Increased incidence of undifferentiated type of gastric cancer with tumor progression in 912 patients with early gastric cancer and 1245 with advanced gastric cancer. Cancer 73:2459–2463

Innocente SA, Abrahamson JLA, Cogswell JP, Lee JM (1999) p53 regulates a G2 checkpoint through cyclin B1. Proc Natl Acad Sci USA 96:2147–2152

Japanese Gastric Cancer Association (1999) Japanese classification of gastric carcinoma, 13th edn. Kanehara, Tokyo

Jares P, Rey MJ, Fernandes PL, Campo E, Nadal A, Munoz M, Mallofre C, Muntane J, Nayach I, Estape J, Cardesa A (1997) Cyclin D1 and retinoblastoma gene expression in human breast carcinoma: correlation with tumor proliferation and oestrogen receptor status. J Pathol 182:160–166

Jin P, Hardy S, Morgan DO (1998) Nuclear localization of cyclin B1 controls mitotic entry after DNA damage. J Cell Biol 141:875–885

Kawamoto H, Koizumi H, Uchikoshi T (1997) Expression of the G2-M checkpoint regulators cyclin B1 and cdc2 in nonmalignant and malignant human breast lesions. Immunocytochemical and quantitative image analyses. Am J Pathol 150:15–23

Korenaga D, Takesue F, Yasuda M, Honda M, Nozoe T, Inutsuka S, Sugimachi K (2002) The relationship between a cyclin B1 overexpression and lymph node metastasis in human colorectal cancer. Surgery 131:114–120

Kushner J, Bradley G, Young B, Jordan RCK (1999) Aberrant expression of cyclin A and cyclin B1 proteins in oral carcinoma. J Oral Pathol Med 28:77–81

Li J, Meyer AN, Donoghue DJ (1997) Nuclear localization of cyclin B1 mediates its biological activity and is regulated by phosphorylation. Proc Natl Acad Sci USA 94:502–507

Mashal RD, Lester S, Corless C, Richie JP, Chandra R, Propert KJ, Dutta A (1996) Expression of cell cycle-regulated proteins in prostate cancer. Cancer Res 56:4159–4163

Molendini J, Benassi MS, Magagnoli G, Merli M, Sollazzo MR, Ragazzini P, Gamberi G, Ferrari C, Balladelli A, Bacchini P, Picci P (1998) Prognostic significance of cyclin expression in human osteosarcoma. Int J Oncol 12:1007–1011

Moriguchi S, Kamakura T, Odaka T, Nose Y, Maehara Y, Korenaga D, Sugimachi K (1991) Clinical features of the differentiated and undifferentiated types of advanced gastric carcinoma: univariate and multivariate analyses. J Surg Oncol 48:202–206

Murakami H, Furihata M, Ohtsuki Y, Ogoshi S (1999) Determination of the prognostic significance of cyclin B1 overexpression in patients with esophageal squamous cell carcinoma. Virchows Arch 434:153–158

Nishida N, Fukuda Y, Komeda T, Kita R, Sando T, Furusawa M, Amenomori M, Shibagaki I, Nakao K, Ikenaga M, Ishizaki K (1994) Amplification and overexpression of the cyclin D1 gene in aggressive human hepatocellular carcinoma. Cancer Res 54:3107–3110

Park M, Chae HD, Yun J, Jung M, Kim YS, Kim SH, Han MH, Shin DY (2000) Constitutive activation of cyclin B1-associated cdc2 kinase overrides p53-mediated G2-M arrest. Cancer Res 60:542–545

Patel V, Jakus J, Harris CM, Ensley JF, Robbins KC, Yeudell WA (1997) Altered expression and activity of G1/S cyclins and cyclin-dependent kinases characterize squamous cell carcinomas of the head and neck. Int J Cancer 73:551–555

Pines J, Hunter T (1990) Human cyclin A is adenovirus E1A-associated protein p60 and behaves differently from cyclin B. Nature 346:760–763

Roncalli M, Basari S, Marchetti A, Buttitta F, Bossi P, Graziani D, Peracchia A, Bonavina L, Viale G, Coggi G (1998) Cell cycle-related gene abnormalities and product expression in esophageal carcinoma. Lab Invest 78:1049–1057

Sakaguchi T, Watanabe A, Sawada H, Yamada Y, Yamashita J, Matsuda M, Nakajima M, Miwa T, Hirao T, Nakano H (1998) Prognostic value of cyclin E and p53 expression in gastric carcinoma. Cancer 82:1238–1243

Sherr CJ (1996) Cancer cell cycles. Science 274:1672–1677

Soria JC, Jang SJ, Khuri FR, Hassan K, Liu D, Hong WK, Mao L (2000) Overexpression of cyclin B1 in early-stage non-small cell lung cancer and its clinical implication. Cancer Res 60:4000–4004

Taylor WR, Stark GR (2001) Regulation of the G2/M transition by p53. Oncogene 20:1803–1815

Toyoshima F, Moriguchi T, Wada A, Fukuda M, Nishida E (1998) Nuclear export of cyclin B1 and its possible role in the DNA damage-induced G2-checkpoint. EMBO J 17:2728-2735

Volm M, Koomagi R, Rittgen W (1998) Clinical implication of cyclins, cyclin-dependent kinases, Rb and E2F1 in squamous-cell lung carcinoma. Int J Cancer 79:294–299

Wang A, Yoshimi N, Ino N, Takuji T, Mori H (1997) Overexpression of cyclin B1 in human colorectal cancers. J Cancer Res Clin Oncol 123:124–127

Yasui W, Kuniyasu H, Yokozaki, Semba S, Shimamoto F, Tahara E (1996) Expression of cyclin E in colorectal adenomas and adenocarcinomas: correlation with expression of Ki-67 and p53 protein. Virchows Arch 429:13–19