

# Expression of E-cadherin and $\beta$ -catenin in gastric carcinoma and its correlation with the clinicopathological features and patient survival

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## Abstract

**AIM:** The E-cadherin-catenin complex is important for cell-cell adhesion of epithelial cells. Impairment of one or more components of this complex is associated with poor differentiation and increased invasiveness of carcinomas. We evaluated the expression pattern of E-cadherin and  $\beta$ -catenin in gastric carcinoma and dysplasia and analyzed their relationship with tumor clinicopathological features and patient survival.

**METHODS:** Immunohistochemical staining of E-cadherin and  $\beta$ -catenin was performed from paraffin specimens of 163 gastric carcinomas, 44 gastric mucosal dysplasia, and 25 intestinal metaplasia, 28 atrophic gastritis and 12 healthy controls.

**RESULTS:** Normal membrane staining was observed in intestinal metaplasia, atrophic gastritis and control biopsy specimens for E-cadherin and  $\beta$ -catenin. 36 % and 16 % of gastric dysplasia were stained abnormally for E-cadherin and  $\beta$ -catenin respectively. Abnormal expression of E-cadherin and  $\beta$ -catenin was demonstrated in 46 % and 44 % of gastric carcinoma respectively. Abnormal expression of E-cadherin and  $\beta$ -catenin occurred more significantly in Borrmann III/IV than in Borrmann I/II type ( $P < 0.005$ , respectively). A significantly higher proportion of signet-ring, mucinous and tubular adenocarcinomas were abnormally expressed for E-cadherin and  $\beta$ -catenin as compared with papillary adenocarcinomas ( $\chi^2 = 8.47, P < 0.005$ , and  $\chi^2 = 7.05, P < 0.01$ , respectively). Moreover, abnormal E-cadherin and  $\beta$ -catenin staining occurred more frequently in diffuse than in intestinal type of tumor ( $\chi^2 = 18.18$  and  $17.79, P < 0.005$ , respectively). There was a significant correlation between abnormal  $\beta$ -catenin expression and positive lymph node metastasis. A survival advantage was noted in tumors retaining normal membranous expression of  $\beta$ -catenin, independent of type, grade, or stage of the disease ( $P < 0.0005$ ).

**CONCLUSION:** Abnormal expression of the E-cadherin-catenin complex occurs frequently in gastric carcinoma, closely related to its histogenesis. Abnormal expression of the E-cadherin-catenin complex in gastric dysplasia may be an early event in the tumorigenesis. The close correlation with poor survival suggests that abnormal  $\beta$ -catenin may be a useful prognostic marker.

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## INTRODUCTION

It is well-known that anchorage of cells to substrate is critical for the integrity of many cell types including epithelial cells. The cadherins are a major class of adhesion molecules which play an important role in the homotypic cell-cell adhesion and hence cancer cell metastasis and invasion. E-cadherin is a member of the cadherin family which is expressed in all epithelial cells<sup>[1,2]</sup>. It is a calcium-dependent cell adhesion molecule that binds to other E-cadherin molecules on adjacent cells and is located at the adhesion junction of epithelial cells<sup>[1]</sup>. Functional cadherin-dependent cell adhesion requires the formation of complexes between E-cadherin and the cytoplasmic proteins known as the catenins. The catenin, which interacts with the cytoplasmic domain of E-cadherin, consists of at least three proteins:  $\alpha$ -,  $\beta$ -, and  $\gamma$ -catenin.  $\beta$ -catenin forms a complex with E-cadherin, while  $\alpha$ -catenin links this complex to the actin cytoskeleton<sup>[3,4]</sup>. It is postulated that changes in cell-cell and cell-matrix interactions account for the ability of cancer cells to cross normal tissue boundaries and metastasize<sup>[5]</sup>. In addition, loss of cell adhesion may contribute to loss of contact inhibition and thus play a role at the earlier stage of the neoplastic process.

Previous studies have provided evidence that perturbation of E-cadherin-mediated cell adhesion is involved in tumor progression and metastasis<sup>[6,7]</sup>. Loss of E-cadherin expression or function *in vitro* has been associated with loss of differentiation and increased invasive capacity of cancer cell lines<sup>[8,9]</sup>. A variety of human cancers exhibit altered expression of E-cadherin that correlates with high grade and advanced tumor stage<sup>[10-12]</sup>. These changes may result from deletions, point mutations of the E-cadherin genes or CpG methylation of the promoter region.

$\beta$ -catenin appears to bind directly and most tightly to E-cadherin and so affects the strength of cell-cell adhesion<sup>[13]</sup>. It is also involved in the Wntless/Wnt signaling pathway and interacts with epidermal growth factor receptor<sup>[14,15]</sup>, with the APC tumor suppressor gene, and with two novel nuclear transcription factors, T-cell factor (TCF)-4 and lymphoid enhancing factor (LEF)-1<sup>[16,17]</sup>.

Gastric cancer is the second most common malignancy worldwide, and is among the leading causes of mortality in countries such as Japan, China, and Chile. Even in the developed Western countries, the 5-year survival rate for gastric cancer is only 10-19 %<sup>[18]</sup>. In China, gastric cancer is the most common malignancy diagnosed annually, with a high cancer-related mortality (25 %)<sup>[19]</sup>. Among the highly prevalent geographic areas of gastric cancer is Lanzhou, a city of northwest China. It is currently unknown what factors contribute to the development, progression, and metastasis of gastric cancers in this geographic area<sup>[20,21]</sup>.

Reduced or completely lost E-cadherin-catenin complex expression has been found in gastric, although results regarding the degree of aberrant E-cadherin-catenin complex expression and its relationship with clinicopathological features and patient survival are contradictory. In this study, by using the immunohistochemical staining, we investigated the expression pattern of E-cadherin or  $\beta$ -catenin in gastric cancers, dysplasia, intestinal metaplasia, atrophic gastritis and normal gastric mucosa and the possible role of E-cadherin-catenin complex in the pathogenesis of gastric cancers in Lanzhou area. The possible relationship between the expression of the E-cadherin and  $\beta$ -catenin and the tumor clinicopathology, as well as its potential value in the evaluation of patient survival were also discussed.

## MATERIALS AND METHODS

### *Patients and tissue samples*

The specimens of gastric cancers were obtained from 185 consecutive patients who underwent gastrectomy at the Department of Surgery (First Teaching Hospital, Lanzhou Medical College, Lanzhou, P. R. China) between January 1995 and December 1996. None of the patients received chemotherapy or radiotherapy before surgery. Clinicopathological information and survival data were obtained from hospital records and patients' doctor in charge. Six patients who died within 4 weeks following surgery were excluded from the survival analysis for the purpose of eliminating bias caused by surgical operation-related death. In sixteen cases, suitable well-preserved blocks could not be obtained, thus a total of 163 patients were included in the final study. There were 123 males and 40 females (3.08:1), with median age of 54.5 years (range, 28-77 years). Samples were taken from the representative cancerous lesions as well as adjacent non-cancerous mucosa. In addition, mucosal biopsy specimens from 32 patients with dysplasia (plus 12 cases from gastrectomy, total 44 cases), 25 with intestinal metaplasia, 28 with atrophic gastritis and 12 healthy controls were examined.

For microscopic examination, tissues were routinely fixed with formalin before being embedded in paraffin. A 4- $\mu$ m section from each specimen block was stained with H & E for histological evaluation, and representative blocks were chosen for immunohistochemical study. In addition, normal colonic epithelium was used as positive control and adjacent normal gastric mucosa as internal control. Negative controls included adjacent section of the same block in which the primary antibody was replaced by phosphate-buffered saline (PBS).

### *Tumor staging and classification*

Tumors were staged at the time of surgery by the standard criteria for TNM staging using the unified international gastric cancer staging classification<sup>[22]</sup> and the following morphological details were recorded: depth of invasion (pT category), lymph node involvement (pN category). By Lauren system<sup>[23]</sup>, tumors were classified into intestinal and diffuse

types. According to the criteria of WHO classification, tumors were classified into adenocarcinoma, which was defined as papillary adenocarcinoma, tubular adenocarcinoma, mucinous adenocarcinoma and signet-ring cell carcinoma, and undifferentiated carcinoma. The gross appearance of tumors was diagnosed using the Borrmann's classification. Dysplasia was diagnosed according to the Padova International Classification<sup>[24]</sup> for gastric dysplasia.

### *Antibodies and other chemicals*

The following items were purchased from Maxim Biotech (Maxim Biotech Inc., South San Francisco, CA, USA): mouse monoclonal antibodies against human E-cadherin and  $\beta$ -catenin; UltraSensitive S-P Kit; and peroxidase-conjugated streptavidin. Diaminobenzidine tetrahydrochloride (DAB) and other routine chemicals were obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA).

### *Immunohistochemical staining*

To detect the presence and patterns of E-cadherin and  $\beta$ -catenin, we utilized the peroxidase-conjugated streptavidin immunostaining technique, as previously described by others<sup>[25]</sup>. Briefly, 4  $\mu$ m tissue sections were dewaxed and rehydrated through changes of xylene and graded alcohol, then to water. Endogenous peroxidase activity was blocked by incubating the sections with 0.6 % hydrogen peroxide for 10 min. Heat-mediated antigen retrieval was performed by heating the sections (immersed in 0.01M citrate buffer, pH 6.0) in a microwave oven (750 W) for 20 min. The slides were then washed with PBS before being exposed to 10 % normal goat serum for 10 min to block the non-specific background reaction. The slides were then incubated with respective primary antibody overnight at 4 °C. Following washes with PBS, the slides were incubated for 15 min with the secondary antibody, biotinylated goat anti-mouse IgG. The slides were further washed for 3 $\times$ 10 min in PBS, followed by incubation with peroxidase-conjugated streptavidin for 10 min. The peroxidase reaction was developed in PBS using hydrogen peroxide as substrate and DAB as a chromogen. Sections were counterstained with haematoxylin, dehydrated, and evaluated under a light microscope.

### *Evaluation of immunostaining*

Slides were independently examined by two experienced pathologists who were blinded to the stage of the tumor and to the initial score of the other observer, and a high level of concordance (90 %) was achieved. In case of disagreement, the slides were reviewed and a consensus view achieved. Staining intensity was graded semiquantitatively from 0 to 3, as previously described<sup>[25,26]</sup>. 0: negative staining; 1: cytoplasmic staining; 2: heterogeneous staining (tumors composing of both normal and abnormal staining areas); and 3: a normal membranous staining. Because the staining pattern sometimes varied within the same tumor particularly when the differentiation status varied, the final score was based on the dominant pattern. For the ease of data analysis, all tumors with loss of membranous expression were classified as abnormal which included those with absent, heterogeneous or cytoplasmic staining patterns.

### *Statistical analysis*

The correlation between the expression of the E-cadherin or  $\beta$ -catenin and clinicopathological features was analysed for statistical significance by the  $\chi^2$  (chi-square) test. Survival curves were constructed according to the method of Kaplan

and Meier. For differences between curves, the *P* value was calculated using the log rank test. A multivariate Cox regression analysis was performed to assess the prognostic significance of different staining patterns and their relationship with other pathological variables. A *P* value of less than 0.05 was considered as statistically significant. All statistical analyses were performed using the SPSS 8.0 statistical package (USA).

## RESULTS

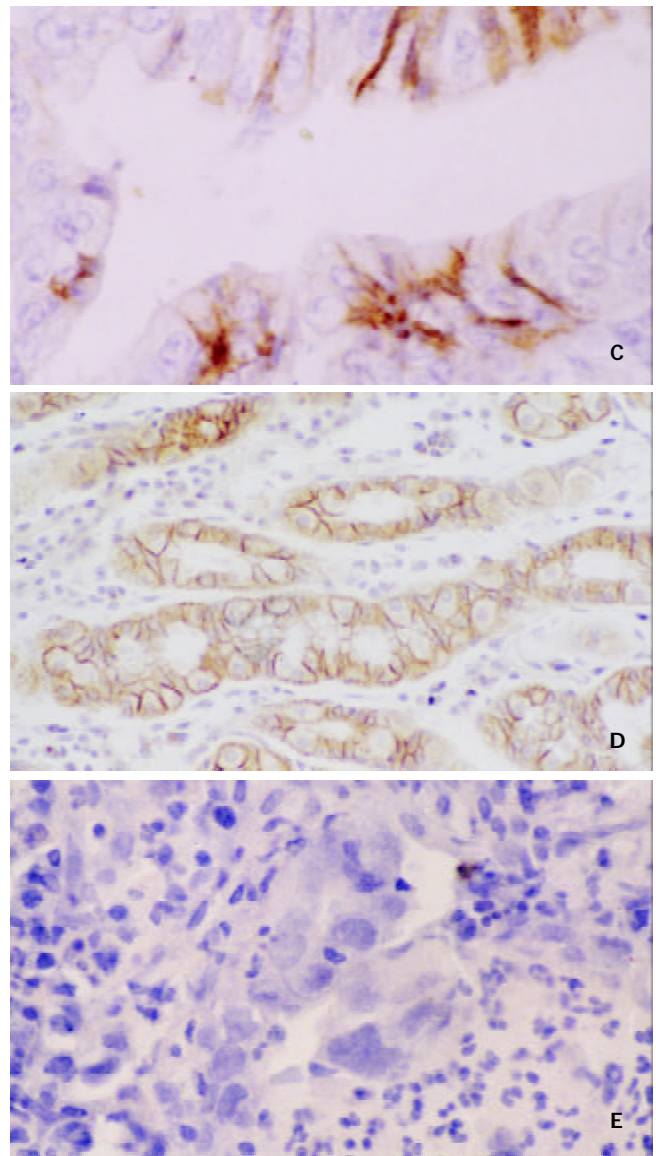
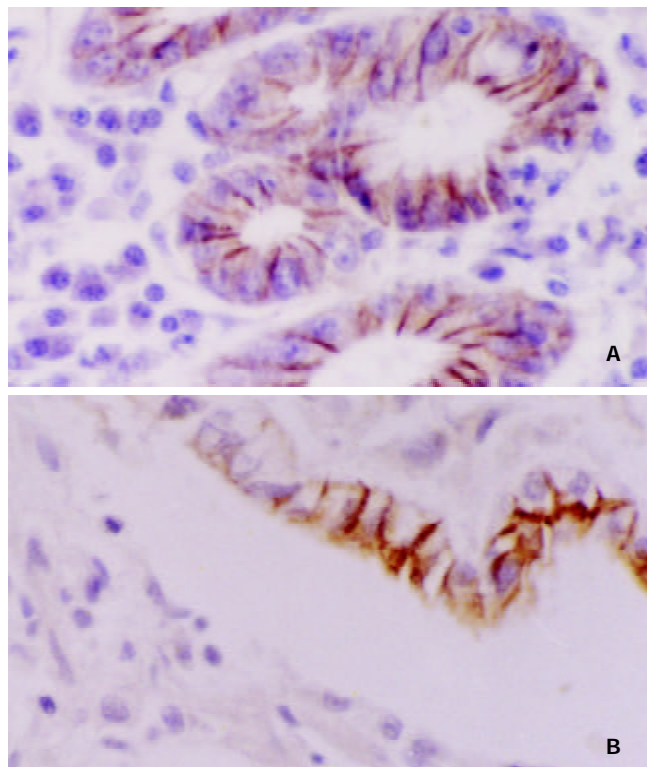
### *Immunohistochemical staining of E-cadherin and b-catenin*

**Normal gastric mucosa** Gastric mucosa specimen from 12 normal health controls were examined. E-cadherin and  $\beta$ -catenin stained intensely in a membranous distribution throughout the epithelium in all the normal control cases (Figure 1).

**Atrophic gastritis, intestinal metaplasia, dysplasia and gastric cancer** Areas of atrophic gastritis, intestinal metaplasia showed normal membranous distribution of staining for E-cadherin and  $\beta$ -catenin in all cases. Abnormal expression was shown in 36.4 % (16/44) of dysplasia for E-cadherin, and in 15.9 % (7/44) for  $\beta$ -catenin. Of the total of 163 primary tumors examined, the abnormal expression rate of E-cadherin and  $\beta$ -catenin were 46.0 % and 44.8 %, respectively (Table 1). Representative examples were shown in Figure 1.

**Table 1** Abnormal expression of E-cadherin and  $\beta$ -catenin in gastric carcinoma and precancerous condition

	Total n	E-cadherin n (%)	$\beta$ -catenin n (%)
Gastric carcinoma	163	75 (46.0)	73 (44.8)
Dysplasia	44	16 (36.4)	7 (15.9)
Intestinal metaplasia	25	0	0
Atrophic gastritis	28	0	0
Health controls	12	0	0



**Figure 1** Immunoreactivity of E-cadherin and  $\beta$ -catenin in Gastric Carcinoma. A. Normal membranous staining pattern of E-cadherin (SP $\times$ 200); B. Poorly differentiated tumor with complete loss of membranous staining with E-cadherin in the majority of cells. Membranous staining can be seen in a few cells retaining gastric gland (SP $\times$ 400); C. Moderately differentiated tumor showing cytoplasmic staining with  $\beta$ -catenin, partly nuclear  $\beta$ -catenin expression (SP $\times$ 400); D. Staining of E-cadherin showing preserved strong membranous expression in gastric mucosa with intestinal metaplasia (SP $\times$ 200); E. Mucosal biopsy specimen with dysplasia (high grade) showing loss of membranous expression of E-cadherin. (SP $\times$ 400)

### *Expression of E-cadherin or b-catenin and gross appearance in gastric cancer*

The frequencies of abnormal expression in tumors less than 5 cm, 5.1-10 cm and more than 10 cm in length were 33.3 %, 35.5 %, and 74.4 % for E-cadherin, respectively, and 28.2 %, 36.2 %, and 78.9 % for  $\beta$ -catenin, respectively (Table 2). The rate of abnormal expression in tumors more than 10 cm was significantly higher than that in tumors less than 10 cm in length (*P*<0.005, respectively). As shown in Table 2, analysis of 139 cases of gastric cancer (24 cases were excluded from 163 cases because of incomplete surgical record) revealed that the expression of E-cadherin and  $\beta$ -catenin were significantly

correlated with the Borrmann classification: Abnormal expression occurred more significantly often in Borrmann III and Borrmann IV than in Borrmann I and Borrmann II type ( $P < 0.005$ , respectively).

**Table 2** Expression of E-cadherin or  $\beta$ -catenin and Gross Appearance

	No	E-cadherin expression (%)		$\beta$ -catenin expression (%)	
		Abnormal	P value	Abnormal	P value
Tumor size (cm)	163				
0-4.9	48	16 (33.3)		14 (28.2)	
5.0-9.9	76	30 (35.5)	16.58	29 (36.2)	21.41
$\geq 10.0$	39	29 (74.4)	$< 0.005^a$	30 (78.9)	$< 0.005^a$
Gross appearance	139				
Borrmann I	12	1 (8.3)		2 (16.7)	
Borrmann II	16	3 (18.8)		3 (18.8)	
Borrmann III	80	35 (43.8)	11.92	32 (40.0)	9.65
Borrmann IV	31	21 (67.7)	$< 0.005^b$	24 (77.4)	$< 0.005^b$

<sup>a</sup> $\geq 10.0$  group vs 0-4.9 or 5-9.9 group; <sup>b</sup>Borrmann III and Borrmann IV groups vs Borrmann I and Borrmann II groups

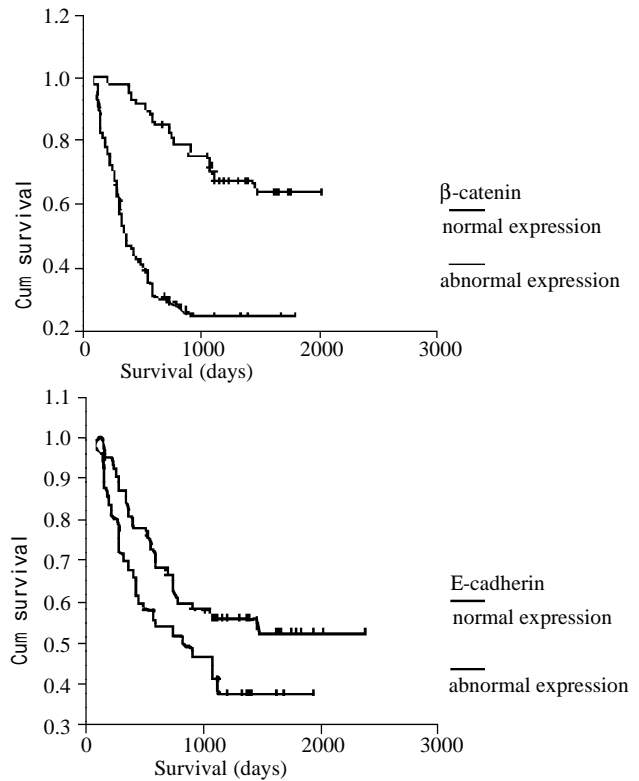
**Expression of E-cadherin or  $\beta$ -catenin and histopathological findings**

Table 3 shows no correlation could be verified between expression of E-cadherin or  $\beta$ -catenin and the invasion depth of the tumor ( $T_1/T_2$  vs  $T_3/T_4$ ). Among the 163 cases of gastric cancers in our series, lymphatic metastasis was observed in 92 cases (56.4 %). We found that there was no correlation between the abnormal expression of E-cadherin and the positivity of lymphatic metastasis. However, the expression of  $\beta$ -catenin was closely correlated with lymphatic metastasis ( $P < 0.005$ ).

According to the WHO classification, papillary adenocarcinoma showed abnormal expression of E-cadherin and  $\beta$ -catenin more frequently compared with signet-ring carcinoma and mucinous and tubular adenocarcinoma ( $P < 0.005$  and  $P < 0.01$ , respectively). Furthermore, according to the histological classification of Lauren, abnormal expression of E-cadherin and  $\beta$ -catenin was significantly higher in diffuse than in intestinal types ( $P < 0.005$ , respectively).

**Survival analysis**

Survival data were obtained for 113 of the patients. Kaplan-Meier curves were computed to compare survival of patients with normal versus abnormal expression of E-cadherin and  $\beta$ -catenin. The overall median survival of patients in this study was 1067 days (range 78-2365). Average survival time for patients showing abnormal expression of E-cadherin was  $1065 \pm 118$  days, normal expression of E-cadherin was  $1508 \pm 121$  days. Patients with normal expression of E-cadherin appeared to have a longer survival time compared with those who have abnormal E-cadherin expression, although the difference was not statistically significant ( $P = 0.058$ ). The average survival time for patients with abnormal expression of  $\beta$ -catenin was  $764 \pm 110$  days, as opposed to the survival time of  $1798 \pm 105$  days for patients with normal expression ( $P < 0.0005$ ) (Figure 2). A multivariate Cox regression analysis confirmed statistically significant association of abnormal  $\beta$ -catenin expression with poor survival ( $P < 0.0005$ ), independent of tumor type, grade, or stage.



**Figure 2** Kaplan-Meier survival curves showing a statistically significant survival advantage found in all tumors with normal membranous expression of  $\beta$ -catenin (B) compared with those with loss of membranous expression ( $P < 0.0005$ ). Abnormal E-cadherin (A) staining pattern showed a trend toward worse survival, but not statistically significant ( $P = 0.058$ ).

**Table 3** Relationship Between Expression of E-cadherin or  $\beta$ -catenin and Histopathological Features in Gastric Carcinoma

	No	E-cadherin expression (%)		$\beta$ -catenin expression (%)	
		Abnormal	P value	Abnormal	P value
pT category					
$T_1/T_2$	65	28 (43.1)		29 (44.6)	
$T_3/T_4$	98	47 (48.0)	NS	44 (44.9)	NS
pN category					
N-negative	71	29 (40.8)		20 (28.2)	14.56
N-positive	92	46 (50.0)	NS	53 (57.6)	$< 0.005$
WHO classification					
Adenocarcinoma	160				
Papillary	16	3 (18.8) <sup>a</sup>		2 (7.1) <sup>a</sup>	
Tubular	109	48 (44.0)		46 (42.2)	
Mucinous	24	13 (54.2)	8.47	15 (62.5)	7.05
Signet ring	11	8 (72.7)	$< 0.005$	7 (63.3)	$< 0.01$
Undiff. carcinoma	3	3 (100)		3 (100)	
Lauren classification					
Intestinal type	108	36 (33.3) <sup>b</sup>	18.18	34 (31.5) <sup>b</sup>	17.79
Diffuse type	40	29 (72.5)	$< 0.005$	28 (70.0)	$< 0.005$
Mixed type	15	10 (66.7)		11 (73.3)	

<sup>a</sup>Papillary vs Tubular, Mucinous and Signet ring; <sup>b</sup>Intestinal type vs Diffuse type. NS: No Significance

## DISCUSSION

Aberrant expression of E-cadherin and  $\beta$ -catenin with loss of membranous localization has been detected in a number of human cancers. Previous studies<sup>[27-30]</sup> have shown variable abnormal expression of E-cadherin-catenin complex ranging from 20 % up to 90 % of tumors. In gastric carcinoma or other human carcinomas, the overall expression of E-cadherin-catenin complex as reported in different studies is difficult to compare due to different score systems and different antibodies used. In this study, 46 % of gastric carcinomas showed abnormal E-cadherin expression and 44 % showed abnormal  $\beta$ -catenin expression, so our data are in line with previous reports which revealed alteration of E-cadherin and  $\beta$ -catenin expression in gastric carcinomas. In addition, by using the Kaplan-Meier curve and A multivariate Cox regression analysis, we found that  $\beta$ -catenin but not E-cadherin expression abnormalities were associated with poor survival, confirming the findings of Jawhari *et al*<sup>[25]</sup> and Ramesh *et al*<sup>[48]</sup>. However, the use of E-cadherin as a patient survival indicator needs to be further verified, as previous findings by Gabbertz *et al*<sup>[30]</sup>, Shun *et al*<sup>[29]</sup>, and Grabsch *et al*<sup>[31]</sup> indicated that there was correlation between abnormal E-cadherin expression and patient survival in gastric carcinoma.

We demonstrated a significant correlation between expression of E-cadherin-catenin complex and tumor differentiation. Aberrant E-cadherin or  $\beta$ -catenin expression was associated with diffuse-type (poorly differentiated) carcinomas. Similarly, abnormal expression rate of E-cadherin and  $\beta$ -catenin was significantly higher in low- and undifferentiated adenocarcinomas than in well-differentiated adenocarcinomas. Previous *in vitro* studies<sup>[8]</sup> have shown that loss of E-cadherin in human carcinoma cell lines is associated with poor differentiation and fibroblastoid morphology. Furthermore, down-regulation of E-cadherin protein product after E-cadherin-specific antisense RNA transfection results in an invasive and de-differentiated phenotype<sup>[9]</sup>. Transfection of E-cadherin cDNA into undifferentiated carcinoma cells produces an epithelioid morphology and increases intercellular adhesion. These effects are reversible using anti-E-cadherin antibodies and provide direct *in vitro* evidence of the crucial role of E-cadherin in the regulation of cell polarity, cellular differentiation, and epithelial morphology<sup>[9,32]</sup>. Our results support the above findings, suggesting that E-cadherin-catenin complex may play an important role in the genesis of histological differentiation and may be used as a differentiation marker in gastric carcinoma<sup>[33,34]</sup>.

The E-cadherin-mediated cell adhesion system is known to act as an "invasive suppressor system"<sup>[24,35,36]</sup> and tumors with reduced E-cadherin expression were also reported to have a higher frequency of lymph node involvement, distant metastasis and morphologic degree of invasiveness than those with preserved E-cadherin expression<sup>[37,38]</sup>. In this study, decreased E-cadherin expression occurred more frequently in tumors of more infiltrative growth on gross appearance or in those of larger size than in their counterparts, but showed no association with the invasion depth of the tumor, and lymph node metastasis. This findings strongly suggests that the presence of E-cadherin as revealed by immunohistochemistry might not indicate that E-cadherin is necessarily functional. For instance, certain mutations in the E-cadherin gene or changes in E-cadherin associated cytoplasmic proteins, the catenin, may alter their adhesive functions. Mutations affecting intercellular adhesion mechanisms have emerged as important steps in the development and progression of many human epithelial tumors. In gastric carcinomas, mutations of the E-

cadherin gene have been reported<sup>[39-42]</sup>. A germline mutation in E-cadherin associated with familial gastric carcinoma was recently reported in a New Zealand kindred<sup>[43]</sup>. Moreover, *in vitro* studies with E-cadherin-negative carcinoma cell lines suggested that methylation around the promoter region CpG island may be one mechanism of E-cadherin inactivation in human carcinomas<sup>[44,45]</sup>. Catenins have been classified into  $\alpha$ ,  $\beta$ , and  $\gamma$ . The cytoplasmic tail of E-cadherin interacts with either  $\beta$ - or  $\gamma$ -catenins which bridge E-cadherin to the cytoskeleton through  $\alpha$ -catenin<sup>[46,47]</sup>. Many studies indicate that the association of E-cadherin with catenins is essential for their cell-cell adhesion<sup>[25,48]</sup>. Mutations in  $\alpha$ - and  $\beta$ -catenins have not been convincingly demonstrated<sup>[49]</sup>, but protein expression abnormalities are relatively frequent, and occur in both diffuse and intestinal carcinomas<sup>[50,51]</sup>. As the function of E-cadherin is modulated by  $\alpha$ -catenin, loss of  $\alpha$ -catenin expression or deletion of  $\alpha$ -catenin could suppress E-cadherin-mediated cell-cell adhesion activity, despite normal E-cadherin and  $\beta$ -catenin expression<sup>[52]</sup>.  $\beta$ -catenin is a multifunctional protein, and plays an important role in Wnt/Wingless signal transduction in addition to its function as a cell-adhesion components<sup>[53]</sup>. Posttranslational modification of  $\beta$ -catenin molecule by phosphorylation has been shown to disrupt the interaction between E-cadherin and  $\alpha$ -catenin, causing loss of E-cadherin-dependent intercellular adhesiveness and in turn potentiating the neoplastic process<sup>[53,54]</sup>. Furthermore, the association of abnormal  $\beta$ -catenin expression with a worse survival, independent of tumor type, grade, or stage, seems to suggest that it acts as an independent prognostic variable.

The pathogenesis of gastric carcinoma remains largely unknown. Although a number of genetic and molecular alterations have been described in gastric carcinoma<sup>[55-57]</sup>, the exact sequence and number of genetic or molecular alterations are not yet known. There were several patterns postulated on how histomorphological changes of the gastric mucosa will progress to the development of gastric carcinoma. The best known was proposed by Correa *et al*<sup>[58]</sup> who described the development of gastric tumors from gastritis through intestinal metaplasia, dysplasia, and eventually to gastric carcinoma. Recent *in vitro* and *in vivo* studies have shown that alteration or loss of E-cadherin expression seems to be critical for the development of gastric carcinomas. Furthermore, Ohene *et al*<sup>[59]</sup>, Xiangming *et al*<sup>[60]</sup>, Blok *et al*<sup>[61]</sup> and Shun *et al*<sup>[62]</sup> reported that abnormal E-cadherin-catenin complex expression occurred in early gastric carcinomas and changes in E-cadherin expression might be early events in gastric carcinoma. Our analysis aimed at investigating E-cadherin-catenin complex expression in the possible precancerous conditions, such as atrophic gastritis, intestinal metaplasia and dysplasia and evaluating the role of E-cadherin-catenin complex expression in the development of gastric carcinoma. In this study, we found that abnormal expression was shown in 36.4 % of dysplasia for E-cadherin and 16 % for  $\beta$ -catenin, respectively, while atrophic gastritis, intestinal metaplasia showed normal expression. Our observations of decreased E-cadherin and  $\beta$ -catenin expression in dysplasia raises the possibility that changes in the E-cadherin-catenin complex occur at an early stage in the neoplastic process.

In conclusion, abnormal expression of E-cadherin and  $\beta$ -catenin occurs in a considerable proportion of gastric carcinomas and correlates with loss of differentiation. Loss of normal  $\beta$ -catenin expression showed a close correlation with poor survival, independent of tumor type, grade, and stage, suggesting that it may be an independent prognostic marker. Our results also indicate that changes in abnormal expression of E-cadherin-catenin complex may be early events in gastric carcinomas.

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