Prognostic Significance of E-Cadherin/Catenin Complex Expression in Gastric Cancer

Abnormal expression of E-cadherin/catenin complex in cancer has been associated with poor differentiation and acquisition of invasiveness, suggesting a possible role of this protein as an invasion suppressor. In this study, we conducted an immunohistochemical investigation of all components of the E-cadherin/catenin complex in 65 gastric cancer patients. Abnormal expression of E-cadherin and, α- and γ-catenin occurred more frequently in diffuse than in intestinal type of gastric cancer, and correlated with poor differentiation. Abnormal expression of E-cadherin and β-catenin correlated with poor survival. Abnormal expression of all four components of the complex was associated with poorly differentiated and diffuse-type carcinoma, and poor survival. In the multivariate analysis, abnormal expression of the E-cadherin/catenin complex was not an independent prognostic factor. These results suggest that the E-cadherin/catenin complex may be a useful marker of differentiation and prognosis in gastric cancer. Further studies are warranted to clarify the impact of the E-cadherin/catenin complex on prognostic factor of gastric cancer.

Key Words : Cadherins; Cytoskeletal Proteins; Stomach Neoplasms; Immunohistochemistry; Neoplasm Staging; Prognosis; Survival Analysis

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INTRODUCTION

Invasion and metastasis of cancers are complex processes, of which the initial step is the escape of cancer cells from primary tumor involving disruption of normal cell-cell adhesion (1). E-cadherin is expressed on the cell surface in most epithelial tissues and is important for establishing cell polarity and maintaining epithelial integrity and cellular differentiation (2). Since E-cadherin is thought to play a major role in the intercellular adhesion of cancer cells, there has been increasing interest in the structure and function of these and other molecules that mediate cell-cell adhesion.

E-cadherin is a 120-kDa transmembrane glycoprotein that is responsible for calcium-dependent intercellular adhesion by homotypic interactions (2-4). Formation of strong intercellular adhesion requires a linkage between E-cadherin and actin filaments in the cytoskeleton. The extracellular domain of E-cadherin contains several Ca^{2+}-binding domains, which self-associate and cause intercellular adhesion. Special intracellular proteins such as α-, β- and γ-catenins are required to link the cytoplasmic domains of E-cadherin to the cytoskeletal elements (5-7). E-cadherin interacts directly with β- or γ-catenin, whereas α-catenin binds indirectly to E-cadherin via other catenins and mediates the connection of the E-cadherin cell adhesion complex with the actin skeleton (2, 5-7). Such binding is essential for the adhesive function of E-cadherin and for the establishment of tight physical cell-cell adhesion. A number of studies have documented the absolute necessity of interaction of E-cadherin with the catenins for normal adhesive function (5-8). Impairment of cadherin-mediated adhesion is likely to constitute one of the main factors leading to the reduced cell-cell adhesion characteristic of neoplastic cells. Since the participation of catenins is essential for the process of cadherin-mediated adhesion in neoplastic cells, catenins, like cadherins, may present various types of alterations that affect adhesive functions of cadherins and contribute to epithelial carcinogenesis. E-cadherin has been studied extensively in relation to its role in cancer invasion and is often down regulated in various cancers (9-13). Since the interaction of E-cadherin with the catenins is essential for intercelluar adhesion, examination of all components of the E-cadherin/catenin complex in cancer tissue is important to evaluate the changes in intercellular adhesion in cancer. To date, little is known about the immunoreactivity of all components of the E-cadherin/catenin complex in gastric cancer.

The aim of this study is to evaluate the expression of

all components of the E-cadherin/catenin complex in gastric cancer tissue by immunohistochemistry, and to examine the relationship of their expression with various clinicopathological parameters and patient survival.

MATERIALS AND METHODS

Patients and tumor specimens

Formalin-fixed, paraffin-embedded tissue blocks were obtained from 65 patients who underwent surgery for gastric cancer during 1992 at Chonnam National University Hospital. Blocks were selected by review of original pathologic slides and all included the junction between normal gastric epithelium and tumor. Patient characteristics including sex, age at the time of surgery, histologic grade, stage and survival data were obtained by medical records. The histologic grade were classified according to the criteria of Lauren and the World Health Organization (14, 15). The tumors were staged at the time of surgery by the standard criteria for TNM staging using the American Joint Committee on Cancer (16).

Antibodies

Monoclonal mouse immunoglobulin antibodies to E-cadherin and α-, β-, and γ-catenin were purchased from Zymed Laboratories Inc. (South San Francisco, CA, U.S.A.). The primary antibodies were diluted in phosphate-buffered saline supplemented with 5% normal horse serum and 1% bovine serum albumin. Final dilutions were as follows: anti-E-cadherin; 1:50, anti-α-catenin; 1:100, anti-β-catenin; 1:100, anti-γ-catenin; 1:50.

Immunohistochemistry

All procedures for immunohistochemical staining were done by Micro-Probe staining system (Fisher Scientific, Pittsburgh, PA, U.S.A.) based on capillary action and by the avidin-biotin peroxidase complex method (17). Paraffin sections, 4 μm in thickness, mounted on Probe-on slides were deparaffinized and heated in microwave oven for 7 min to retrieve the antigens. They were immersed in 0.6% hydrogen peroxide for 5 min to block the endogenous peroxidase activity. The primary antibodies were treated for 120 min at room temperature. Anti-mouse immunoglobulin G (Sigma, St. Louis, MO, U.S.A.) labeled with biotin was added, and the samples were incubated for 7 min at 45°C. After multiple rinses with universal buffer, streptavidin-alkaline phosphatase detection system (Biomeda, Foster, CA, U.S.A.) was applied for 7 min. As the final step, the slides were developed

for 20 min with the enzyme substrate 3 amino-9-ethyl carbazole (AEC, Sigma, St. Louis, MO, U.S.A.). The slides were counterstained with hematoxylin solution for 1 min (Research Genetics, Huntsville, AL, U.S.A.). After washing and dehydration, the slides were sealed with a universal mount (Research Genetics).

Evaluation of E-cadherin and catenins expression

Assessment of the staining was done by two independent observers without knowledge of clinical outcomes such as tumor stage, grade and survival. Consensus scores were assigned for each case by reviewing the slides with discrepancies in scoring. All sections on which the two observers disagreed were re-evaluated and discussed for appropriate classification. The expression of E-cadherin and catenins in cancer cells was compared to that of normal epithelial cells in the same sample.

In accordance with previously published criteria (11-13), cancer cells which immunostained as strongly as normal epithelial cells were defined as positive. E-cadherin and catenins expression in the tumors was graded according to the proportion of positive cells. When >90% of cancer cells were positively stained, the tumors were considered uniformly positive (+); when 10-90% were positively stained, the tumors were considered to be heterogenous (±); when 0-10% of the cells were positively stained, the tumors were considered to be negative (–). Uniformly positive staining patterns were regarded as normal, while uniformly negative and heterogenous stainings were considered as abnormal expression.

Statistical analysis

The χ^2-test and Fisher's exact test, where appropriate, were used to compare the expression of the E-cadherin/catenin complex with various clinicopathological parameters. Actuarial survival rates of patients with normal or abnormal E-cadherin/catenin complex expression were evaluated according to the Kaplan-Meier method and the differences were tested with a log-rank test. The Cox regression model was used to determine the prognostic significance of each parameter by a multivariate analysis. The statistical software program used was Statistical Package for the Social Sciences (SPSS/PC+ 8.0, Chicago, IL, U.S.A.). A p-value of less than 0.05 was accepted as statistically significant.

RESULTS

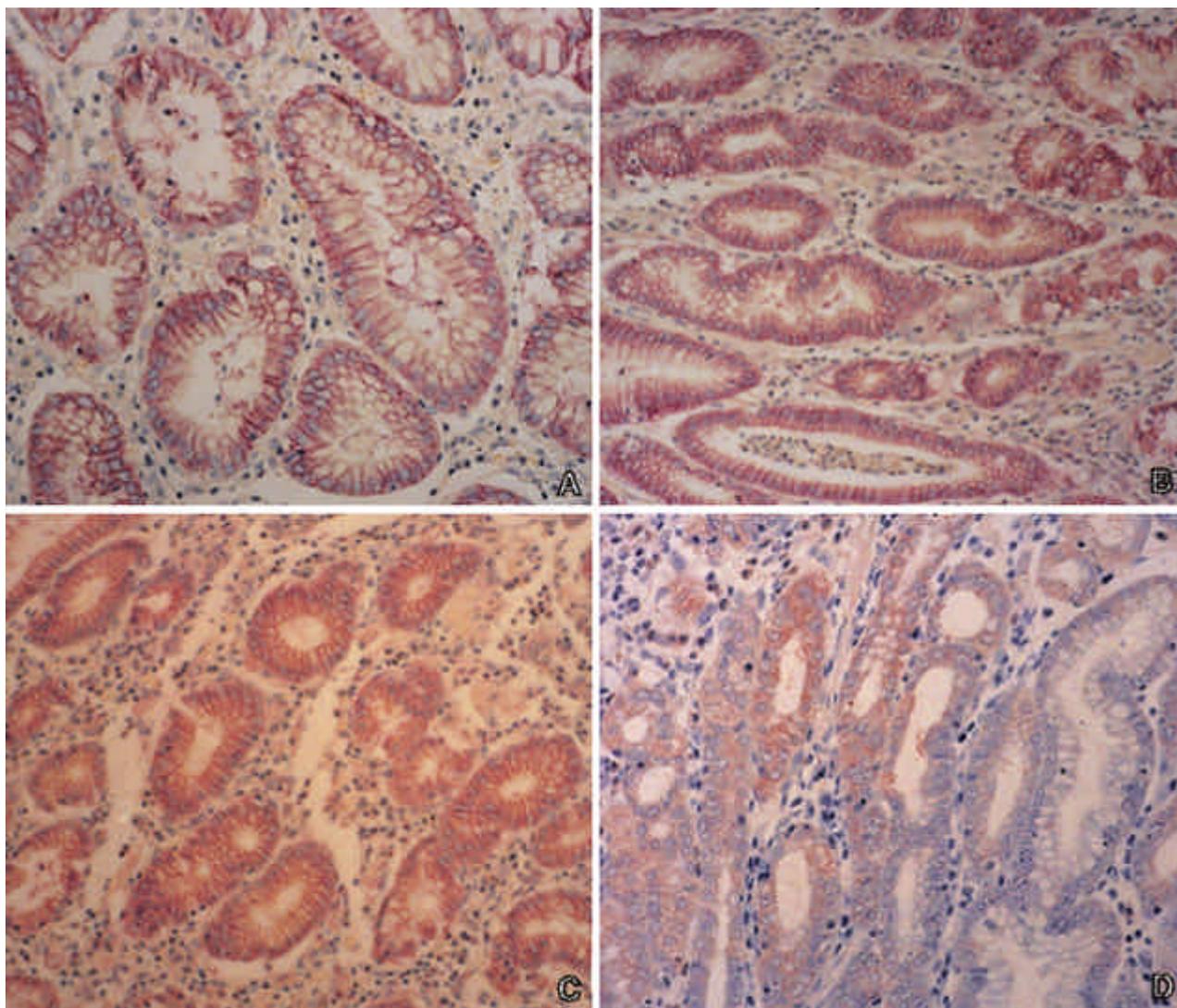This study group comprised 38 males and 27 females.

Fig. 1. Immunoreactivity of E-cadherin (A), α- (B), β- (C) and γ-catenin (D) in normal gastric epithelium. All the epithelial cells express E-cadherin, α-, β- and γ-catenin on cell-cell boundaries ($\times 200$).

The mean age was 55.2 ± 10.3 (mean $\pm$ S.D.) yr with a range from 28 to 73 yr.

Expression of E-cadherin and catenins

In the mucosa of noncancerous areas, epithelial cells showed equally strong membranous expression of E-cadherin, α-, β- and γ-catenin proteins at cell-cell boundaries, reflecting the normal localization of an intercellular adhesion molecule; these served as an internal positive control (Fig. 1). Stromal background or nuclear staining was not found at all. Generally, normal gastric epithelium displayed strong staining for E-cadherin, α-, and β-catenin whilst γ-catenin stained faintly positive. In gastric carcinoma, positive immunoreactivity for E-cadherin, α-, β- and γ-catenin was predominantly associated with cell-cell boundaries as

normal epithelium (Fig. 2, 3). Heterogenous immunoreactivity had a staining with variable degrees of both membrane and cytoplasmic staining. Negative immunoreactivity expressed a trace amount of E-cadherin, α-, β-, and γ- catenin (Fig. 2, 3). Abnormal expression of E-cadherin, α-, β-, and γ-catenin was demonstrated in 52.3, 47.7, 44.6, and 61.5%, respectively (Table 1).

Correlation between expression of E-cadherin and catenins, and clinicopathological parameters

The correlation between E-cadherin expression and clinicopathological parameters is summarized in Table 2. E-cadherin expression did not correlate with the patient's age, sex, stage, depth of invasion, lymph node involvement or distant metastasis. The rate of abnormal expression in tumors larger than 5.5cm was higher than
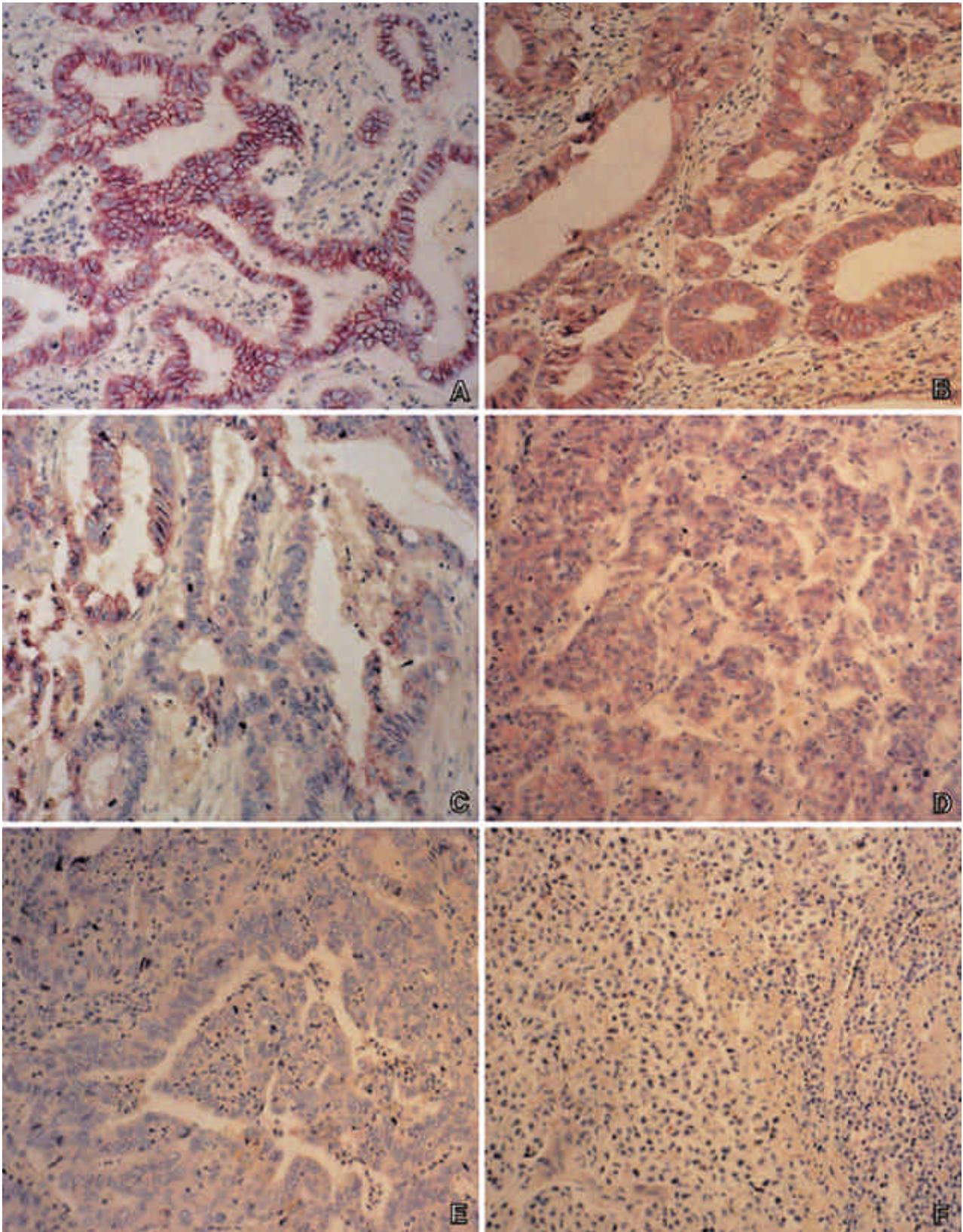
Fig. 2. Immunoreactivity of E-cadherin and β-catenin in gastric adenocarcinomas. **A**, **C**, and **E**, stained for E-cadherin; regarded as +, ±, and −, for E-cadherin expression respectively. **B**, **D** and **F**, stained for β-catenin; regarded as +, ±, and −, for β-catenin expression respectively (×200).

Fig. 3. Immunoreactivity of α-catenin and γ-catenin in gastric adenocarcinomas. A, C, and E, stained for α-catenin; regarded as +, ±, and −, for α-catenin expression respectively. B, D and F, stained for γ-catenin; regarded as +, ±, and −, for γ-catenin expression respectively (×200).
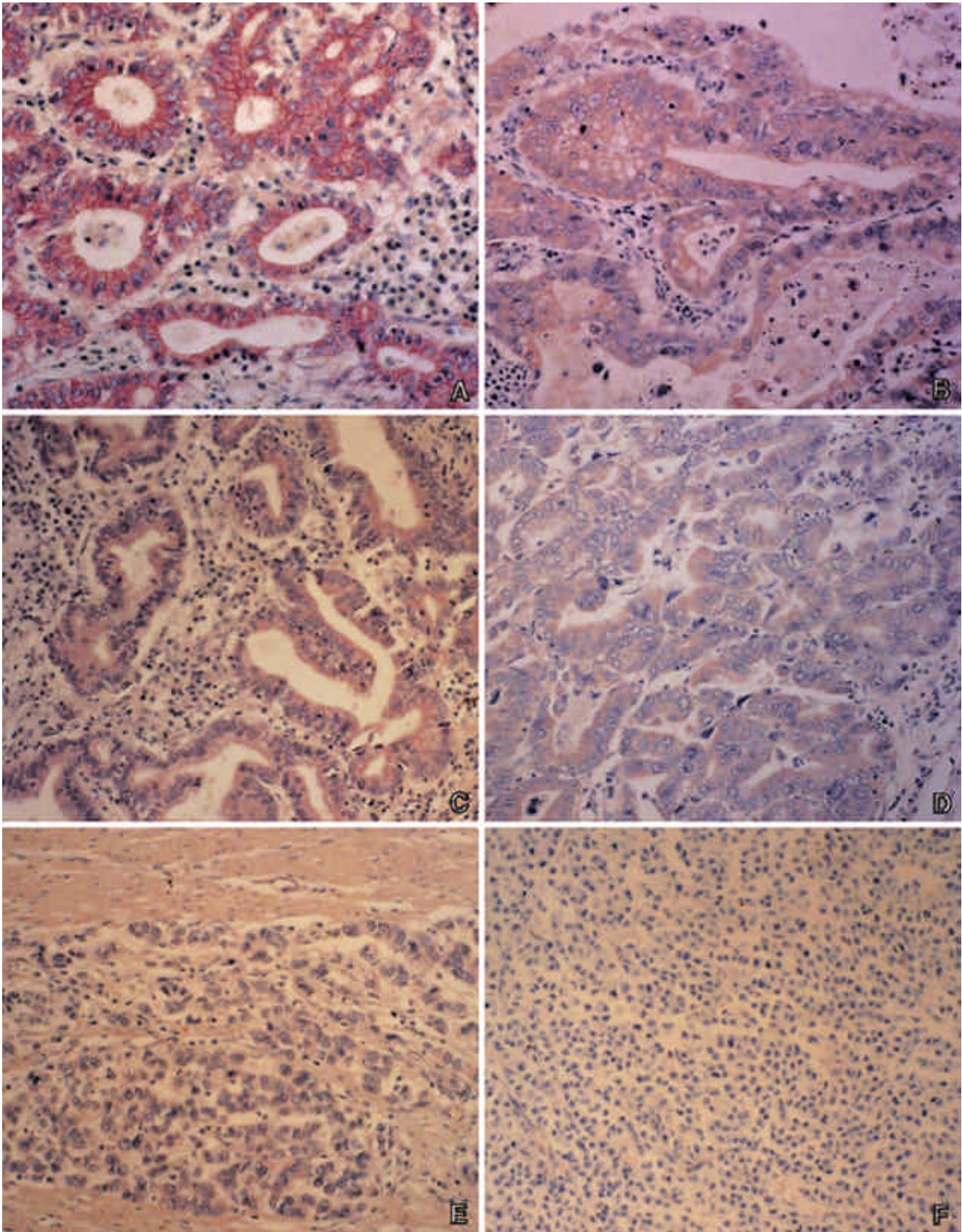
Table 1. Frequency for the grade of expression of each adhesion molecule in 65 gastric cancers

Expression	E-cadherin n (%)	α-catenin n (%)	β-catenin n (%)	γ-catenin n (%)
Normal				
Positive (+)	31 (47.7)	34 (52.3)	36 (55.4)	25 (38.5)
Abnormal (±, −)	34 (52.3)	31 (47.7)	29 (44.6)	40 (61.5)
Heterogenous (±)	23 (35.4)	20 (30.8)	20 (30.8)	23 (35.4)
Negative (−)	11 (16.9)	11 (16.9)	9 (13.8)	17 (26.2)

Table 2. Correlation between E-cadherin expression and clinicopathological parameters of gastric cancer

Clinicopathological parameters	Total (n=65)	E-cadherin expression Normal (n=31) +	E-cadherin expression Abnormal (n=34) ±	E-cadherin expression Abnormal (n=34) −	p value
Age (yr)					
<55	30	14	11	5	0.878
≥55	35	17	12	6	
Sex					
Male	38	16	16	6	0.285
Female	27	15	7	5	
Tumor size (cm)					
<5.5	38	23	11	4	0.022
≥5.5	27	8	12	7	
Lauren classification					
Intestinal	28	19	9	0	0.002
Diffuse	25	5	11	9	
Mixed	12	7	3	2	
Differentiation grade					
Well differentiated	15	11	4	0	<0.001
Moderately differentiated	21	14	7	0	
Poorly differentiated	29	6	12	11	
TNM stage					
I	22	12	7	3	0.683
II	7	4	1	2	
III	19	9	8	2	
IV	17	6	7	4	
Depth of tumor invasion					
T1	8	6	2	0	0.405
T2	16	8	5	3	
T3	29	12	9	8	
T4	12	5	7	0	
Lymph node metastasis					
Absence	27	14	8	5	0.571
Presence	38	17	15	6	
Distant metastasis					
Absence	51	27	16	8	0.106
Presence	14	4	7	3	

+, positive; ±, heterogenous; −, negative immunoreactivity

that in tumors smaller than 5.5 cm ($p=0.022$). According to the histological classification of Lauren, the abnormal expression of E-cadherin was more frequent in diffuse than in intestinal or mixed types of cancer ($p=0.002$). Abnormal expression of E-cadherin correlated significantly with poorly differentiated tumor, significantly ($p<0.001$).

The correlations between α- and γ-catenin expression and clinicopathological parameters are summarized in Table 3 and 5, respectively.

Abnormal expression of α- and γ-catenin occurred more frequently in diffuse than intestinal tumor ($p=0.040$ and 0.022, respectively), and correlated with poor differentiation ($p<0.001$ and 0.001, respectively). α-

Table 3. Correlation between α-catenin expression and clinicopathological parameters of gastric cancer

Clinicopathological parameters	Total (n=65)	α-catenin expression			p value
		Normal (n=34)	Abnormal (n=31)		
		+	±	−	
Age (yr)					
<55	30	16	8	6	0.805
≥55	35	18	12	5	
Sex					
Male	38	18	12	8	0.515
Female	27	16	8	3	
Tumor size (cm)					
<5.5	38	22	12	4	0.215
≥5.5	27	12	8	7	
Lauren classification					
Intestinal	28	20	6	2	0.04
Diffuse	25	8	10	7	
Mixed	12	6	4	2	
Differentiation grade					
Well differentiated	15	14	1	0	<0.001
Moderately differentiated	21	11	9	1	
Poorly differentiated	29	9	10	10	
TNM stage					
I	22	9	10	3	0.11
II	7	5	1	1	
III	19	14	4	1	
IV	17	6	5	6	
Depth of tumor invasion					
T1	8	3	3	2	0.854
T2	16	8	7	1	
T3	29	17	6	6	
T4	12	6	4	2	
Lymph node metastasis					
Absence	27	14	10	3	0.883
Presence	38	20	10	8	
Distant metastasis					
Absence	51	30	15	6	0.061
Presence	14	4	5	5	

+, positive; ±, heterogenous; −, negative immunoreactivity

and γ-catenin expression did not correlate with patient's age, sex, tumor size, stage, depth of invasion, lymph node involvement or distant metastasis.

The correlation between β-catenin expression and clinicopathological parameters is summarized in Table 4.

Abnormal expression of β-catenin is significantly associated with advanced tumor stage ($p=0.020$), and also correlates with distant metastasis ($p=0.006$).

There was a trend towards an association between abnormal expression of β-catenin and, poor differentiation and depth of invasion, although these associations were not statistically significant ($p=0.083$ and 0.076, respectively). β-catenin expression did not correlate with patient's age, sex, tumor size, stage, Lauren classification or lymph node involvement.

Expression of all components of E-cadherin/catenin complex

Sixteen tumors (24.6%) displayed normal expression of all four components of the E-cadherin/catenin complex, while 17 tumors (26.2%) displayed abnormal expression of all four components of the complex (Table 6).

Abnormal expression of all four components of the complex was associated with poorly differentiated and diffuse-type carcinomas ($p=0.003$ and 0.015, respectively).

Survival analysis

The overall patient survival according to the expression

Table 4. Correlation between β-catenin expression and clinicopathological parameters of gastric cancer

| Clinicopathological parameters | Total (n=65) | β-catenin expression | | | p value |
| | | Normal (n=36) | Abnormal (n=29) | | |
		+	±	−	
Age (yr)					
<55	30	16	8	6	0.805
≥55	35	20	12	3	
Sex					
Male	38	19	14	5	0.308
Female	27	17	6	4	
Tumor size (cm)					
<5.5	38	24	11	3	0.205
≥5.5	27	12	9	6	
Lauren classification					
Intestinal	28	19	7	2	0.188
Diffuse	25	12	7	6	
Mixed	12	6	5	1	
Differentiation grade					
Well differentiated	15	12	2	1	0.083
Moderately differentiated	21	11	10	0	
Poorly differentiated	29	13	8	8	
TNM stage					
I	22	15	4	3	0.02
II	7	5	2	0	
III	19	12	6	1	
IV	17	4	8	5	
Depth of tumor invasion					
T1	8	6	2	0	0.076
T2	16	11	2	3	
T3	29	16	9	4	
T4	12	3	7	2	
Lymph node metastasis					
Absence	27	18	6	3	0.138
Presence	38	18	14	6	
Distant metastasis					
Absence	51	33	14	4	0.006
Presence	14	3	6	5	

+, positive; ±, heterogenous; −, negative immunoreactivity

of E-cadherin, α-, β- and γ-catenin in tumor is shown in Fig. 4. Analysis of the survival for all patients showed that abnormal expression of E-cadherin and β-catenin was correlated with poor survival (p=0.0299 and 0.0189, respectively). In contrast, there was no apparent association between poor survival and abnormal expression of α- or γ-catenin (p=0.3007 and 0.7349 respectively). The overall patient survival according to expression of all components of E-cadherin/catenin complex in tumor specimens is shown in Fig. 5. Abnormal expression of all four components of the E-cadherin/catenin complex correlated with poor survival (p=0.0039). However, when E-cadherin/catenin complex status and other clinicopathological parameters were analyzed by the Cox regression model, abnormal expression of the E-cadherin/catenin complex was not found to be an independent prognostic factor (data not shown).

DISCUSSION

In the course of cancer progression, cancer cells must first become detached from the primary lesion, traverse through the basement membrane and then migrate into the extracellular matrix. Cell-cell adhesion molecules play an important role in the initial stage of cancer invasion and metastasis (18, 19).

Since E-cadherin is known to be the prime mediator of cell-cell adhesion and epithelial tissue integrity, these alterations may lead to loss of cell differentiation, al-

Table 5. Correlation between γ-catenin expression and clinicopathological parameters of gastric cancer

| Clinicopathological parameters | Total (n=65) | γ-catenin expression | | | p value |
| | | Normal (n=25) | Abnormal (n=40) | | |
		+	±	−	
Age (yr)					
<55	30	11	10	9	0.804
≥55	35	14	13	8	
Sex					
Male	38	13	15	10	0.403
Female	27	12	8	7	
Tumor size (cm)					
<5.5	38	13	15	10	0.448
≥5.5	27	12	8	7	
Lauren classification					
Intestinal	28	16	11	1	0.022
Diffuse	25	5	7	13	
Mixed	12	4	5	3	
Differentiation grade					
Well differentiated	15	12	2	1	0.001
Moderately differentiated	21	6	14	1	
Poorly differentiated	29	7	7	15	
TNM stage					
I	22	5	10	7	0.281
II	7	3	1	3	
III	19	10	7	2	
IV	17	7	5	5	
Depth of tumor invasion					
T1	8	2	3	3	0.555
T2	16	5	7	4	
T3	29	14	7	8	
T4	12	4	6	2	
Lymph node metastasis					
Absence	27	8	11	8	0.217
Presence	38	17	12	9	
Distant metastasis					
Absence	51	19	18	14	0.703
Presence	14	6	5	3	

+, positive; ±, heterogenous; −, negative immunoreactivity

lowing cells to detach from the primary site and metastasize to lymph nodes and distant organs (20). Loss of differentiation, invasiveness and metastatic potential are often associated with down regulation of E-cadherin expression in many types of human cancer (9-13).

Our study shows a significant reduction of E-cadherin expression in primary gastric carcinomas compared with normal mucosa and a correlation of abnormal E-cadherin expression with poor differentiation. Abnormal E-cadherin expression was associated with poor survival. Many studies have shown loss of E-cadherin expression in human cancers, in general correlated with dedifferantiation and invasive behavior. Other studies, however, have cast some doubt on the importance of E-cadherin expression as a prognostic factor in cancer (21-23). Tumors expressing

E-cadherin normally still fail to form close contacts and cause metastasis (24). These observations suggest that a mechanism which interferes with E-cadherin functioning may exist in such tumors, even in the presence of structurally normal E-cadherin.

E-cadherin participates in the formation of adherens junctions and its function is in part regulated via a carboxy terminal intracellular domain by α-, β- and γ-catenin and cytoskeletal elements of the cells to which they bind (8, 25). Since linkage to the cytoskeleton is required for E-cadherin function, impaired E-cadherin/catenin complex formation or altered catenin expression (26) could account for, at least in part, the finding of normal E-cadherin expression in some tumors.

In vivo studies of a variety of human malignancies in-

Table 6. Correlation between expression of all four components of E-cadherin/catenin complex and clinicopathological parameters of gastric cancer

Clinicopathological parameters	Total (n=33)	Expression of all four components of E-cadherin/catenin complex		p value
		Normal (n=16)	Abnormal (n=17)	
Age (yr)				
<55	13	6	7	0.829
≥55	20	10	10	
Sex				
Male	16	6	10	0.221
Female	17	10	7	
Tumor size (cm)				
<5.5	16	9	7	0.303
≥5.5	17	7	10	
Lauren classification				
Intestinal	11	9	2	0.015
Diffuse	13	3	10	
Mixed	9	4	5	
Differentiation grade				
Well differentiated	7	7	0	0.003
Moderately differentiated	10	5	5	
Poorly differentiated	16	4	12	
TNM stage				
I	10	6	4	0.396
II	3	2	1	
III	11	6	5	
IV	9	2	7	
Depth of tumor invasion				
T1	4	3	1	0.336
T2	8	5	3	
T3	16	7	9	
T4	5	1	4	
Lymph node metastasis				
Absence	12	8	4	0.157
Presence	21	8	13	
Distant metastasis				
Absence	26	15	11	0.085
Presence	7	1	6	

cluding gastric, breast and colorectal adenocarcinomas have shown that loss of function or expression of any of the E-cadherin/catenin complex components is associated with loss of epithelial differentiation and normal tissue architecture and the acquisition of a motile and invasive phenotype (27-29). Our study shows that the expression of α-, β- and γ-catenin was abnormal in 47.7, 44.6, 61.5% of samples, respectively. Abnormal expression of α- and γ-catenin occurred more frequently in diffuse than in intestinal tumor and correlated with poor differentiation. There was a likely association between abnormal expression of β-catenin and poor differentiation, although it was not statistically significant. Although the number of specimens examined was small, all abnormal expression of four components of E-cadherin/catenin complex was associated with poorly differentiated and

diffuse type carcinoma. These findings support the view that expression of the E-cadherin/catenin complex may be a sensitive indicator of cancer differentiation (20).

A number of previous studies have examined the expression of components of the E-cadherin/catenin complex independently, and controversy exists over the correlation between the expression of components of the E-cadherin/catenin complex and various clinicopathological parameters and survival in various human cancers (9-13, 26-29). Our study shows that immunohistochemically detected E-cadherin/catenin complex expression is of minor importance compared with conventional prognostic factors such as stage and status of metastasis, as far as the contribution to invasive and metastatic potential in gastric cancer is concerned.

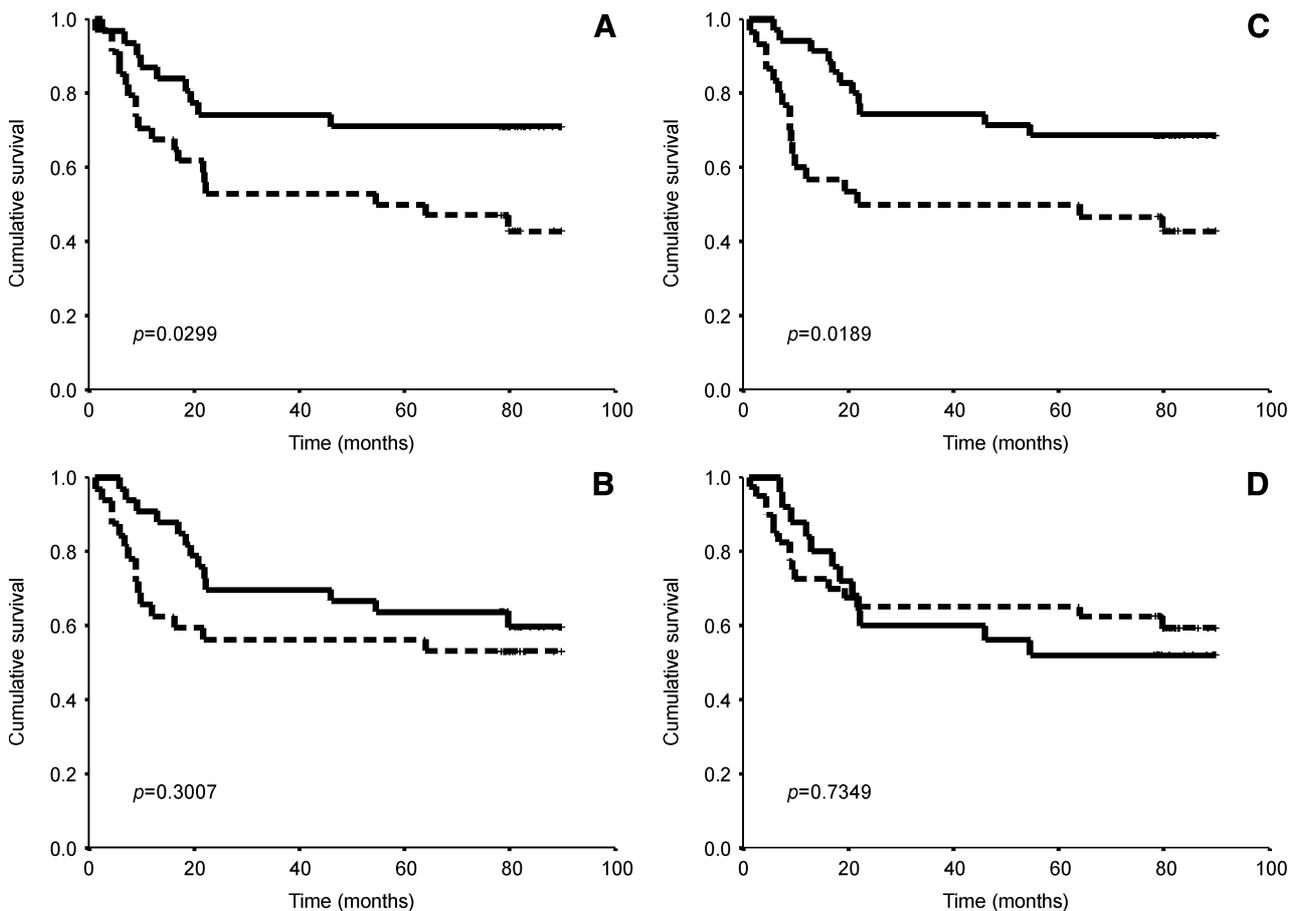These findings support the notion that the invasive

Fig. 4. Kaplan-Meier survival curves correlating survival with normal (solid line) or abnormal (dotted line) expression of E-cadherin (A), α- (B), β- (C), γ-catenin (D). Abnormal expression group of E-cadherin and β-catenin correlate with poor survival.
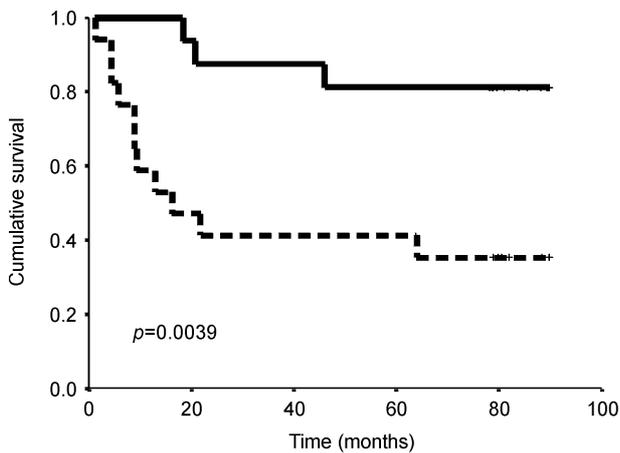

Fig. 5. Kaplan-Meier survival curve correlating survival with all normal (solid line) or abnormal (dotted line) expression of four components of E-cadherin/catenin complex. All abnormal expression of four components of the E-cadherin/catenin complex correlates with poor survival.

and metastatic potential of gastric cancer is not dependent on cell adhesion factors alone, and many other factors including host response are involved. It should also

be noted that immunoreactivity does not provide information about the function of the protein and gene integrity.

In conclusion, we have shown that abnormal expression of the E-cadherin/catenin complex occurs in a considerable proportion of gastric cancer and correlates with loss of epithelial differentiation and normal tissue architecture; unfavorable prognosis is noted only in the univariate analysis, while abnormal expression of the E-cadherin/catenin complex is not an independent prognostic factor in multivariate analysis. These results suggest that the E-cadherin/catenin complex may be a useful marker of differentiation and prognosis in gastric cancer. Further studies are warranted to clarify the impact of the E-cadherin/catenin complex on prognostic factor of gastric cancer.

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