α-catenin expression is decreased in patients with gastric carcinoma

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Reduced α-catenin expression is not correlated with H pylori infection.

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INTRODUCTION

Gastric carcinoma is the second most common malignancy worldwide, and one of the leading causes of death in countries such as Japan, China, and Chile. Even in the developed Western countries, the 5-year survival rate of gastric carcinoma patients is only 10-19%[1]. In China, gastric carcinoma is the most common malignancy annually diagnosed, with a cancer-related mortality of 23%[2]. Lanzhou, a city of northwest China, is one of the highly prevalent geographic areas of gastric carcinoma with a cancer-related mortality of 48%[3]. It is currently unknown what factors contribute to the development, progression, and metastasis of gastric cancers. Many investigations attribute this high incidence to dietary and genetic factors, as well as Helicobacter pylori (H pylori) infection[4].

Development of malignant tumors is in part characterized by the ability of tumor cells to break away from cell-cell adhesion and to invade surrounding tissues. E-cadherin is the main adhesion molecule of epithelia, and has been implicated in carcinogenesis because it is frequently lost in human epithelial carcinomas[5]. Formation of strong intercellular adhesion requires a linkage between transmembranous E-cadherin and actin filaments in the cytoskeleton. The extracellular domain of E-cadherin regulates homophilic interaction. The amino acid sequences of cytoplasmic domain, on the other hand, are highly conserved among the family members and indispensable to bind to actin filaments. At least two cytoplasmic proteins, called α-catenin and β-catenin, are closely associated with this domain[6]. When the carboxyl-terminal region of the cytoplasmic domain of E-cadherin is deleted, the truncated E-cadherin cannot act as adhesion molecules[7,8]. Thus, catenins play an important role in E-cadherin function. α-catenin is believed to be important in linking E-cadherin to the actin cytoskeleton. Biochemical evidence suggests that α-catenin does not bind to E-cadherin directly but rather mediates the interaction of E-cadherin-β-catenin complex with the actin cytoskeleton, whereas β-catenin binds directly
to the cytoplasmic domain of E-cadherin. However, cells lacking α-catenin are unable to form stable adhesions junction, despite normal E-cadherin and β-catenin expression[10-13].

The important role of E-cadherin-catenin complex in mediating epithelial cell-cell adhesion is reflected by many studies showing that its abnormal expression and dysfunction are correlated to cancer cell dedifferentiation, invasion, and metastasis[10-13]. We have previously shown that abnormal expression of E-cadherin and β-catenin occurs in a considerable proportion of gastric carcinomas. In this study, we examined α-catenin expression in gastric carcinoma by reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemical analysis to assess the possible role of α-catenin in the process of gastric carcinogenesis. The possible relationship between α-catenin expression and tumor clinicopathology was also discussed.

MATERIALS AND METHODS

Materials
Specimens of gastric cancers were obtained from 49 consecutive patients who underwent gastrectomy at the Department of Surgery (First Teaching Hospital, Lanzhou Medical College, Lanzhou, China), from January to August 2002. There were 39 males and 10 females, with a median age of 54 years (range, 38-72 years). None of the patients received chemotherapy or radiotherapy before surgery. Clinicopathological information was obtained from hospital records. Samples were taken from the representative cancerous lesions as well as adjacent non-cancerous mucosae. Immediately after removal, all specimens were placed in liquid nitrogen and stored at -80 °C until use. In addition, mucosal biopsy specimens from nine healthy controls were examined. Sections were stained with Warthin-Starry staining for detection of H pylori.

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[14] and the following morphological details were recorded: depth of invasion (pT category), lymph node involvement (pN category). According to the criteria of the Japanese Research Society for Gastric Cancer[14], tumors were classified into well or poorly differentiated (including undifferentiated or signet ring) carcinomas. Pathological data are summarized in Table 1.

RT-PCR assay
Total RNA was extracted by Tripure method (TakaRa, Japan). One microgram of total RNA was reverse transcribed in 20 µL volume of reaction mixture using the SuperScript Preamplification system (TakaRa) according to its instruction. A pair of primers was designed to amplify the coding region of α-catenin mRNA. A sense primer, Pr-1 (5'-TGCGCCA-GCTAGCCGAGAAATG-3'), and an antisense primer, Pr-2 (5'-TCAGCAACCGGTATAACACGAGA-3'), were used and the expected PCR product was 437 bp. PCR was carried out in 25 µL total reaction mixture. Reaction mixture was incubated for 5 min at 94 °C. Samples were denatured at 94 °C for 30 s, annealed at 62 °C for 30 s, and extended at 72 °C for 30 s. After 35 cycles there was a final elongation for 5 min at 72 °C, 10 µL of the PCR product was then electrophoresed on 1.5% agarose gel together with size markers.

Semiquantitative analysis
Levels of α-catenin mRNA were quantified using semiquantitative RT-PCR as previously described. GAPDH was used as an internal standard to confirm the equal loading in each experiment and amplified from the same cDNAs. The level of PCR-amplified products was analyzed densitometrically from the agarose gel and standardized to the respective GAPDH mRNA level. The α-catenin/GAPDH ratio was calculated and analyzed using the t test.

Immunohistochemical staining
The following items were purchased from Maxim Biotech Inc. (South San Francisco, CA, USA): mouse mAbs against human α-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).

To detect the presence and patterns of α-catenin, we utilized the peroxidase-conjugated streptavidin immunostaining technique, as previously described by others[10-13]. Briefly, 4-µm-thick tissue sections were dewaxed and rehydrated through changes of xylene and graded alcohol. 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 0.01 mol/L citrate buffer, pH 6.0) in a microwave oven (750 W) for 20 min. The slides were then washed with PBS before they were 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 the wash with PBS, the slides were incubated for 15 min with the secondary antibody and biotinylated goat anti-mouse IgG. The slides were further washed for 3 min×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 a substrate and DAB as a chromogen. Sections were counter-stained with hematoxylin, dehydrated, and evaluated under a light microscope.

Interpretation of immunostaining
Slides were independently examined by two experienced pathologists who were blinded to the stage of the tumor and

Table 1 Relationship between expression of α-catenin mRNA and histopathological features in gastric carcinoma

| No  | α-catenin mRNA expression | P   | α-catenin mRNA expression | P   |
|-----|---------------------------|-----|---------------------------|-----|
| pT category |                        |     | N category |                        |     |
| T1/T1 | 17                        | 7 (41.2) | 10 (58.8) | 1.36 |
| T2/T1 | 32                        | 8 (25.0) | 24 (75.0) | NS  |
| pN category |                        |     | N category |                        |     |
| N-negative | 26                        | 10 (38.5) | 16 (61.5) | 1.60 |
| N-positive | 23                        | 5 (21.7)  | 18 (78.3) | NS  |
| Tumor grade |                        |     | Differentiated |                        |     |
| 20 | 11 (55.0) | 9 (45.0) | 9.46 |
| Undifferentiated | 29                        | 4 (13.8)  | 25 (86.2) | <0.01 |

*, positive; ±, faint band; -, negative.
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 was achieved. Staining intensity was graded semiquantitatively from 0 to 3, as previously described[14,15]: 0: negative staining; 1: cytoplasmic staining; 2: heterogeneous staining (tumors composed 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 \( \chi^2 \) test was used for correlation between \( \alpha \)-catenin expression and clinicopathologic indices. The correlation between \( \alpha \)-catenin mRNA expression and \( \alpha \)-catenin immunostaining was analyzed by Spearman’s rank correlation coefficient. \( P<0.05 \) was considered statistically significant.

RESULTS
Expression of \( \alpha \)-catenin mRNA in gastric carcinoma
All gastric biopsies obtained from healthy control subjects exhibited \( \alpha \)-catenin mRNA. In contrast, in 34 of 49 tumor samples, \( \alpha \)-catenin mRNA levels altered. Thus, of the 49 gastric tumor tissues, expression of \( \alpha \)-catenin was completely lost in 12 (41%) and decreased in 8 (28%) compared with normal issue (Figure 1). In total, 34 (69%) tumor specimens exhibited low or absent \( \alpha \)-catenin expression, whereas in the tumor-free locations (26 cases) absent \( \alpha \)-catenin expression was observed in only 5 cases.

The relationship between \( \alpha \)-catenin expression and clinicopathological features is shown in Table 1. The frequency of decreased \( \alpha \)-catenin expression was higher in poorly differentiated carcinomas (25 [86%] of 29 cases) than in well-differentiated carcinomas (9 [45%] of 20 cases). Thus, the expression of \( \alpha \)-catenin was significantly correlated with differentiation (\( P<0.01 \)). A significant correlation was not shown between \( \alpha \)-catenin expression and depth of invasion (\( pT \) category), with loss or absent expression in 10 of 17 (59%) tumors with \( T_1/T_2 \) and 24 of 32 (75%) tumors with \( T_3/T_4 \) (\( P>0.05 \)). In comparing the lymph node metastasis (\( pN \) category) with loss or absent expression of \( \alpha \)-catenin, \( \alpha \)-catenin was reduced in 62% (16/26) of tumors with \( N_0 \) and 78% (18/23) of tumors with \( N_1 \) (\( P>0.05 \)).

\( \alpha \)-catenin immunostaining in gastric carcinoma
Without exception, all normal mucosal specimens showed distinct, evenly distributed immunostaining for \( \alpha \)-catenin along the intercellular borders. Of the 49 gastric tumor cases, 35 (71%) showed an abnormal \( \alpha \)-catenin expression, and 14 (29%) showed a normal expression. Furthermore, of the 34 cases whose mRNA expression of \( \alpha \)-catenin was reduced, 32 (94%) showed an abnormal immunostaining pattern, while only 2 showed a normal \( \alpha \)-catenin expression (Figure 2). Using Spearman’s rank correlation coefficient, there was an apparent association between reduced expression of \( \alpha \)-catenin mRNA and abnormal \( \alpha \)-catenin immunostaining (\( r_s=0.867, P=0.001 \)). Comparison of \( \alpha \)-catenin immunostaining with clinicopathological features revealed that abnormal \( \alpha \)-catenin expression correlated with differentiation (\( P<0.01 \)), but not with \( pT \) category or \( pN \) category.

Correlation of \( \alpha \)-catenin expression with \( H \) pylori infection
A total of 49 gastric carcinomas were examined for \( H \) pylori infection using Warthin-Starry staining. \( H \) pylori infection and mRNA expression of \( \alpha \)-catenin were studied. Twenty-two of 31 tissue specimens with \( H \) pylori infection exhibited a reduced \( \alpha \)-catenin expression, whereas in 18 tissue specimens with no \( H \) pylori infection 12 exhibited a low or no \( \alpha \)-catenin expression. A statistically significant correlation was not observed between reduced expression of \( \alpha \)-catenin mRNA and \( H \) pylori infection (\( P>0.05 \)).

DISCUSSION
Functional cell-cell contacts play a critical role in the organization
of differentiated epithelial tissues. It is predominantly regulated by E-cadherin, a calcium-dependent cell adhesion molecule that binds to other E-cadherin molecules on adjacent cells and is located at the adheren junction of epithelial cells\textsuperscript{[3,4,5]}. It has become apparent that loss of E-cadherin-mediated cell-cell adhesion is associated with the progression of many carcinomas, known to act as an “invasion suppressor system” in cancer cells\textsuperscript{[4,10,20]}. In cultured cell lines, loss of E-cadherin function caused a reduction in cell-cell adhesiveness and acquisition of an invasive capacity\textsuperscript{[21,22]}. Also, the reduced expression of E-cadherin has been correlated with differentiation, tumor invasion, and metastasis in various cancerous tissues\textsuperscript{[7,8]}. Loss of E-cadherin/catenin-dependent cell-cell adhesion was often caused by mutations of the E-cadherin or the \( \alpha \)-catenin gene\textsuperscript{[9,23-26]}. Approximately 50\% of sporadic diffuse gastric carcinomas demonstrated somatic mutations that could inactivate E-cadherin\textsuperscript{[27,28]}. A germline mutation in E-cadherin associated with familial gastric carcinoma was recently reported in a New Zealand kindred\textsuperscript{[29,30]}. Another mechanism of altered cell-cell adhesion is the downregulation of E-cadherin gene expression, for instance by modified methylation patterns of a \( 5\)'CpG island in the E-cadherin promoter\textsuperscript{[30-32]}. Moreover, catenins (\( \alpha \), \( \beta \)) play an important role in E-cadherin function. Mutant E-cadherin lacking the catenin-binding sites failed to interact with the actin filaments\textsuperscript{[7,8]}, indicating that interactions between E-cadherin and cytoskeletal proteins through catenins could confer stability on the cell-cell adhesion junctions. Recently, several studies\textsuperscript{[16,33-35]} have shown that expression of E-cadherin and catenins is frequently downregulated in gastric carcinoma. We have previously demonstrated that abnormal expression of E-cadherin and \( \beta \)-catenin occurs in a considerable proportion of gastric carcinomas, and there is a significant relationship between abnormal E-cadherin or \( \beta \)-catenin expression and tumor clinicopathology.

\( \alpha \)-catenin, a protein associated with E-cadherin, could bind to the cytoplasmic domain of E-cadherin, form a linkage to actin filaments, and regulate E-cadherin function\textsuperscript{[56,57]}. PC9 lung carcinoma cells, which do not have the \( \alpha \)-catenin protein, could not bind tightly to each other despite the fact that they possess E-cadherin. Transfection of the \( \alpha \)-catenin gene could restore their intercellular adhesion\textsuperscript{[29]}. As no methylation around the promoter region CpG island has been documented in the \( \alpha \)-catenin gene\textsuperscript{[36]}, gene deletions might be involved in reduced expression of \( \alpha \)-catenin. In addition, post-transcriptional mechanisms may be important in the regulation of \( \alpha \)-catenin function. E-cadherin might stabilize \( \alpha \)-catenin protein through their specific binding to \( \alpha \)-catenin\textsuperscript{[37,38]}, or might increase the translation level of this molecule by decreasing the concentration of free \( \alpha \)-catenin protein\textsuperscript{[39]}. In our study, we found loss of membranous \( \alpha \)-catenin immunostaining in a subgroup of gastric carcinomas, and these results were in agreement with those of previous reports\textsuperscript{[36,37,39,40]}. However, these studies were based on immunohistochemical analyses and alteration of \( \alpha \)-catenin expression was defined as abnormal non-membranous immunostaining in gastric carcinoma cells. In the present study, using a different approach, we found that mRNA expression of \( \alpha \)-catenin was lost or downregulated, compared with the adjacent normal gastric mucosa or normal gastric tissues from healthy subjects. Of the 49 cases studied, \( \alpha \)-catenin expression was found to be downregulated or lost in 69\% of gastric carcinomas. These results suggest that alteration of \( \alpha \)-catenin occurs at the transcription level. We found that reduced \( \alpha \)-catenin expression did not correlate with \( pT \) category or \( pN \) category, but correlated strongly with poor differentiation grade. Thus, our results suggest that \( \alpha \)-catenin expression may be used as a differentiation marker. Moreover, our findings of reduced \( \alpha \)-catenin mRNA expression in 5 of 26 tumor-free gastric mucosa suggest that changes in \( \alpha \)-catenin expression may be an early event in gastric carcinoma.

It has been accepted that patients infected with \( H \) pylori undergo up to ninefold greater risk of developing gastric carcinoma and several groups have reported that \( H \) pylori infection leads to enhanced cell proliferation and diminished apoptosis of the gastric mucosa and both these features are common in the process of malignant transformation. Terres et al\textsuperscript{[41]} reported that \( H \) pylori-infected individuals exhibited downregulation of E-cadherin expression. Furthermore, Yu et al\textsuperscript{[42]}, have previously shown that loss or downregulation of \( \alpha \)-catenin mRNA in the gastric mucosa is associated with \( H \) pylori infection. However, in this study, there was no statistically significant correlation between \( \alpha \)-catenin expression and \( H \) pylori infection. Since the number of carcinoma cases in this study was small, further investigations are required.

In conclusion, loss or absence of \( \alpha \)-catenin expression is common in gastric carcinoma, and may be an early event in gastric carcinoma. \( \alpha \)-catenin expression may be used as a differentiation marker. Reduced \( \alpha \)-catenin expression does not correlate with \( H \) pylori infection.

REFERENCES

1. Hansson LE, Sparen P, Nyren O. Survival in stomach cancer is improving: results of a nationwide population-based Swedish study. Ann Surg 1999; 230: 162-169

2. Sun X, Mu R, Zhou Y, Dai X, Qiao Y, Zhang S, Huangfu X, Sun J, Li L, Lu F. 1990-1992 mortality of stomach cancer in China. Zhonghua Zhongliu Zazhi 2002; 24: 4-8

3. An international association between Helicobacter pylori infection and gastric cancer. The EUROCAST Study Group. Lancet 1993; 341: 1359-1362

4. Hirohashi S. Inactivation of the E-cadherin-mediated cell adhesion system in human cancers. Am J Pathol 1998; 153: 333-339

5. Hinck L, Nathke IS, Papkoff J, Nelson WJ. Dynamics of cadherin/catenin complex formation: novel protein interactions and pathways of complex assembly. J Cell Biol 1994; 125: 1327-1340

6. Kintner C. Regulation of embryonic cell adhesion by the cadherin cytoplasmic domain. Cell 1992; 69: 225-236

7. Takeichi M. Cadherin cell adhesion receptors as a morphogenetic regulator. Science 1991; 251: 1451-1455

8. Jawhari A, Hirohashi S, Kajimoto T, Inagaki S, Nomura K, Kuroki S, et al. Germline mutations in E-cadherin are associated with familial gastric carcinoma. Cancer Res 1996; 56: 4279-4282

9. Pitcher MA, Kikuchi S, Nakamura S, Kuroki S, Hirohashi S, Orange CP. Reduced expression of the E-cadherin/catenin system in cancer cells. Cancer 2002; 94: 1605-1613

10. Shiozaki H, Oka H, Inoue M, Murakami H, Monden M. E-cadherin mediated adhesion system in cancer cells. Cancer 1996; 77: 1605-1613
sion of the cadherin-catenin complex in oesophageal adenocarcinoma correlates with poor prognosis. J Pathol 1997; 182: 331-338

12 Chan AO, Lam SK, Chu KM, Lam CM, Kwok E, Leung SY, Yuen ST, Law SY, Hui WM, Lai KC, Wong CY, Hu HC, Lai CL, Wong J. Soluble E-cadherin is a valid prognostic marker in gastric carcinoma. Gut 2001; 48: 808-811

13 Popov Z, Gil-Diez de Medina S, Lefrere-Belda MA, Hoznek A, Bastuji-Garin S, Abbou CC, Thiery JP, Radvanyi F, Chopin DK. Low E-cadherin expression in bladder cancer at the transcriptional and protein level provides prognostic information. Br J Cancer 2000; 83: 209-214

14 Kennedy BJ. The unified international gastric cancer staging classification. Scand J Gastroenterol 1987; 22: 11-13

15 Kajitani T. The general rules for the gastric cancer study in surgery and pathology. Part I. Clinical classification. Jpn J Surg 1981; 11: 127-139

16 Jawhari A, Jordan S, Poole S, Browne P, Pignatelli M, Farthing MJ. Abnormal immunoreactivity of the E-cadherin-catenin complex in gastric carcinoma: relationship with patient survival. Gastroenterology 1997; 112: 46-54

18 Ohene-Abuakwa Y, Noda M, Perenyi M, Kobayashi N, Kashima K, Hattori T, Pignatelli M. Expression of the E-cadherin/catenin (alpha-, beta-, and gamma-) complex correlates with the macroscopic appearance of early gastric cancer. J Pathol 2000; 192: 433-439

19 Pignatelli M, Vessey CJ. Adhesion molecules: novel molecular tools in tumor pathology. Hum Pathol 1994; 25: 849-856

19 Lee JH, Koh JT, Shin BA, Ahn KY, Roh JH, Kim YJ, Kim KK. Comparative study of angiostatic and anti-invasive gene expressions as prognostic factors in gastric cancer. Int J Oncol 2001; 18: 355-361

20 Machado JC, Soares P, Carneiro F, Rocha A, Beck S, Blin N, Bera G, Sobrinho-Simoes M. E-cadherin gene mutations provide a genetic basis for the phenotypic divergence of mixed gastric carcinomas. Lab Invest 1999; 79: 459-465

21 Fri xen UH, Behrens J, Sachs M, Eberle G, Voss B, Warda A, Lochner D, Birchmeier W. E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. J Cell Biol 1991; 113: 173-185

22 Vleminkcx K, Vakaet L, Mareel M, Fiers W, van Roy F. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. Cell 1991; 66: 107-119

23 Fukudome Y, Yanagihara K, Takeichi M, Ito F, Shibamoto S. Characterization of a mutant E-cadherin protein encoded by a mutant gene frequently seen in diffuse type gastric carcinoma. Int J Cancer 2000; 88: 579-583

24 Chun YS, Lindor NM, Smyrk TC, Petersen BT, Burgart LJ, Guilford PJ, Donohue JH. Germline E-cadherin gene mutations: is prophylactic total gastrectomy indicated? Cancer 2001; 92: 181-187

25 Zheng ZH, Sun XJ, Qiu GR, Liu YH, Wang MX, Sun KL. E-cadherin gene mutation in precancerous condition, early and advanced stages of gastric cancer. Shijie Huaren Xiaohua Zazhi 2002; 10: 153-156

26 Shimoyama Y, Nagafuchi A, Fujita S, Gotoh M, Takeichi M, Tsukita S, Hirohashi S. Cadherin dysfunction in a human cancer cell line: possible involvement of loss of alpha-catenin expression in reduced cell-cell adhesiveness. Cancer Res 1992; 52: 5770-5774

27 Becker KF, Atkinson MJ, Reich U, Becker I, Nekarda H, Siewert JR, Hoﬄer H. E-cadherin gene mutations provide clues to diffuse type gastric carcinomas. Cancer Res 1994; 54: 3845-3852

28 Machado J, Carneiro F, Sobrinho-Simoes M. E-cadherin mutations in gastric carcinoma. J Pathol 2000; 191: 466-468

29 Guilford P, Hopkins J, Harraway J, McLeod M, McLeod N, Harawira P, Taiie H, Scoular R, Miller A, Reeve AE. E-cadherin germline mutations in familial gastric cancer. Nature 1998; 392: 402-405

30 Tamura G, Yin J, Wang S, Fleisher AS, Zou T, Abraham JM, Kong D, Smolinski KN, Wilson KT, James SP, Silverberg SG, Nishizuka S, Terashima M, Motoyama T, Meltzer SJ. E-Cadherin gene promoter hypermethylation in primary human gastric carcinomas. J Natl Cancer Inst 2000; 92: 569-573

31 Leung WK, Yu J, Ng EK, To KE, Ma PK, Lee TL, Go MY, Chung SC, Sung JJ. Concurrent hypermethylation of multiple tumour-related genes in gastric carcinoma and adjacent normal tissues. Cancer 2001; 91: 2294-2301

32 Rountree MR, Bachman KE, Baylin SB. DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. Nat Genet 2000; 25: 269-277

33 Karatas G, Karayiannakis AJ, Syrigos KN, Chatziigianni E, Papamikoulaou S, Simatos G, Papamikoulaou D, Bogris S. Expression patterns of the E-cadherin-catenin cell-cell adhesion complex in gastric cancer. Hepatogastroenterology 2000; 47: 1465-1469

34 Joo YE, Rew JS, Kim HS, Choi SH, Park CS, Kim SJ. Changes in the E-cadherin-catenin complex expression in early and advanced gastric cancers. Digestion 2001; 64: 111-119

35 Blok P, Craenen ME, Dekker W, Tytgat GN. Loss of E-cadherin expression in early gastric cancer. Histopathology 1999; 34: 410-415

36 Jubb AM, Bell SM, Quirke P. Methylation and colorectal cancer. J Pathol 1999; 185: 111-134

37 Watabe M, Nagafuchi A, Tsukita S, Takeichi M. Induction of polarized cell-cell association and retardation of growth by activation of the E-cadherin-catenin adhesion system in a dispersed carcinoma line. J Cell Biol 1994; 127: 247-256

38 Nagafuchi A, Takeichi M, Tsukita S. The 102 kd cadherin-associated protein: similarity to vinculin and posttranscriptional regulation of expression. Cell 1991; 65: 849-857

39 Oda T, Kanai Y, Oya T, Yoshiura K, Shimoyama Y, Birchmeier W, Sugimura T, Hirohashi S. E-cadherin gene mutations in human gastric carcinoma cell lines. Proc Natl Acad Sci USA 1994; 91: 1858-1862

40 Shun CT, Wu MS, Lin MT, Chang MC, Lin JT, Chuang SM. Immunohistochemical evaluation of cadherin and catenin expression in early gastric carcinomas: correlation with clinicopathologic characteristics and Helicobacter pylori infection. Oncology 2001; 60: 339-345

41 Terres AM, Pajares JM, O’Toole D, Ahern S, Kelleher D. H pylori infection is associated with downregulation of E-cadherin, a molecule involved in epithelial cell adhesion and proliferation control. J Clin Pathol 1998; 51: 410-412

42 Yu J, Ebert MP, Miehlik S, Rost H, Lendeckel U, Leodolter A, Stolte M, Bayerdorffer E, Malfertheiner P. alpha-catenin expression is decreased in human gastric cancers and in the gastric mucosa of first degree relatives. Gut 2000; 46: 659-664