

• BRIEF REPORTS •

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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Abstract

AIM: To assess the expression of α -catenin in gastric carcinoma and to determine the role of α -catenin expression in gastric carcinogenesis.

METHODS: α -catenin expression was assessed by semi-quantitative reverse transcriptase-polymerase chain reaction and immunohistochemical staining in 49 gastric carcinomas, 26 adjacent non-cancerous mucosae, and gastric biopsy specimens from 11 healthy controls.

RESULTS: mRNA levels of α -catenin were reduced or absent in 34 of 49 (69%) gastric carcinoma tissues and in 5 of 26 (19%) tumor-free gastric mucosae of carcinoma patients, respectively. Of the carcinoma samples with altered α -catenin mRNA levels, α -catenin expression was negative in 20 and decreased in 14 cases. Up to 69% of tumors were stained abnormally for α -catenin. Of the 34 cases whose mRNA expression of α -catenin was reduced, 32 (94%) showed abnormal immunostaining patterns, while only 2 showed a normal α -catenin expression. The frequency of reduced expression of α -catenin mRNA was 14% in well-differentiated carcinomas, higher than that in poorly differentiated carcinomas (86%). A significant correlation was not shown between α -catenin expression and both depth of invasion and lymph node metastasis. Moreover, there was no statistical difference between loss or down-regulation of α -catenin mRNA and *Helicobacter pylori* (*H pylori*) infection.

CONCLUSION: Downregulation of α -catenin expression is common in gastric carcinoma, and α -catenin expression may be used as a differentiation marker. Downregulation of α -catenin expression may be an early event in tumorigenesis.

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%^[2]. 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^[3].

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^[4]. 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^[5,6]. When the carboxyl-terminal region of the cytoplasmic domain of E-cadherin is deleted, the truncated E-cadherin cannot act as adhesion molecules^[5,7,8]. 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^[6,9].

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^[15], tumors were classified into well or poorly differentiated (including undifferentiated or signet ring) carcinomas. Pathological data are summarized in Table 1.

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

	No	α -catenin mRNA expression		P
		+	-or \pm	
All case	49	15 (30.6)	34 (69.4)	
pT category				
T ₁ /T ₂	17	7 (41.2)	10 (58.8)	1.36
T ₃ /T ₄	32	8 (25.0)	24 (75.0)	NS
pN 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; \pm , faint band; -, negative.

RT-PCR assay

Total RNA was extracted by Tripure method (TakaRa, Japan). One microgram of total RNA was reverse transcribed in 20 μ L 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'-TCGCCCCA-GCTAGCCGCAGAAATG-3'), and an antisense primer, Pr-2 (5'-TCAGCAACCGGGTAAACAGCAGAGA-3'), were used and the expected PCR product was 437 bp. PCR was carried out in 25 μ L 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 μ L 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^[13]. Briefly, 4- μ m-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 \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 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

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^[16,17]: 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 χ^2 test was used for correlation between α -catenin expression and clinicopathologic indices. The correlation between α -catenin mRNA expression and α -catenin immunostaining was analyzed by Spearman's rank correlation coefficient. $P < 0.05$ was considered statistically significant.

RESULTS

Expression of α -catenin mRNA in gastric carcinoma

All gastric biopsies obtained from healthy control subjects exhibited α -catenin mRNA. In contrast, in 34 of 49 tumor samples, α -catenin mRNA levels altered. Thus, of the 49 gastric tumor tissues, expression of α -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 α -catenin expression, whereas in the tumor-free locations (26 cases) absent α -catenin expression was observed in only 5 cases.

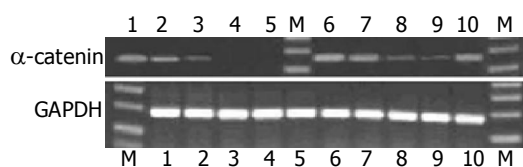


Figure 1 RT-PCR analysis of α -catenin mRNA in gastric tissues. Amplification of GAPDH mRNA was performed to ensure equal loading. Lanes 1 and 10: healthy controls; lane 6: tumor free; lanes 2-5, 7-9: tumor; M: marker.

The relationship between α -catenin expression and clinicopathological features is shown in Table 1. The frequency of decreased α -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 α -catenin was significantly correlated with differentiation ($P < 0.01$). A significant correlation was not shown between α -catenin expression and depth of invasion (pT category), with loss or absent expression in 10 of 17 (59%) tumors with T₁/T₂ and 24 of 32 (75%) tumors with T₃/T₄ ($P > 0.05$). In comparing the lymph node metastasis (pN category) with loss or absent expression of α -catenin, α -catenin was reduced in 62% (16/26) of tumors with N₀ and 78% (18/23) of tumors with N₁ ($P > 0.05$).

α -catenin immunostaining in gastric carcinoma

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

Correlation of α -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 α -catenin were studied. Twenty-two of 31 tissue specimens with *H. pylori* infection exhibited a reduced α -catenin expression, whereas in 18 tissue specimens with no *H. pylori* infection 12 exhibited a low or no α -catenin expression. A statistically significant correlation was not observed between reduced expression of α -catenin mRNA and *H. pylori* infection ($P > 0.05$).

DISCUSSION

Functional cell-cell contacts play a critical role in the organization

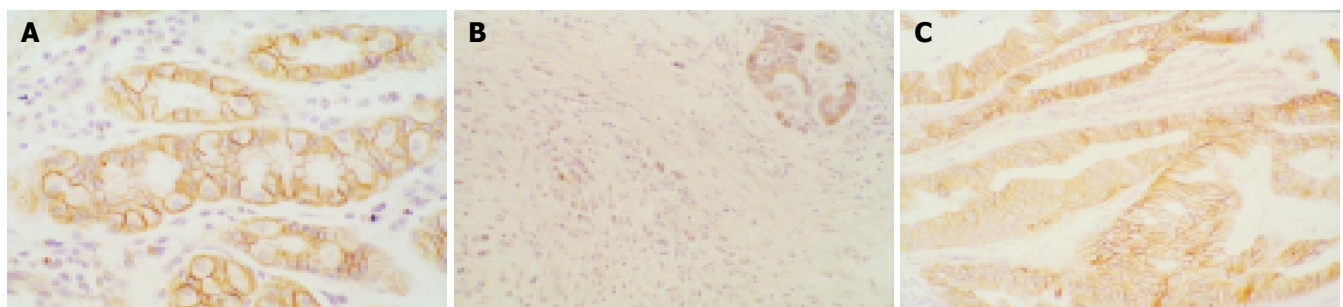


Figure 2 Immunoreactivity of α -catenin in gastric carcinoma. **A:** Normal membranous staining pattern (SP $\times 400$). **B:** Poorly differentiated tumors with complete loss of membranous staining in the majority of cells. Membranous

staining can be seen in a few cells retaining gastric gland (SP $\times 200$). **C:** Moderately differentiated tumors showing membranous staining pattern (SP $\times 200$).

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^[5,18]. It has become now 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^[4,19,20]. In cultured cell lines, loss of E-cadherin function caused a reduction in cell-cell adhesiveness and acquisition of an invasive capacity^[21,22]. Also, the reduced expression of E-cadherin has been correlated with differentiation, tumor invasion, and metastasis in various cancerous tissues^[4,10]. Loss of E-cadherin/catenin-dependent cell-cell adhesion was often caused by mutations of the *E-cadherin* or the α -catenin gene^[9,23-26]. Approximately 50% of sporadic diffuse gastric carcinomas demonstrated somatic mutations that could inactivate E-cadherin^[27,28]. A germline mutation in E-cadherin associated with familial gastric carcinoma was recently reported in a New Zealand kindred^[24,29]. 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^[30-32]. Moreover, catenins (α -, β -) play an important role in E-cadherin function. Mutant E-cadherin molecules lacking the catenin-binding sites failed to interact with the actin filaments^[7,8], indicating that interactions between E-cadherin and cytoskeletal proteins through catenins could confer stability on the cell-cell adheren junctions. Recently, several studies^[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 β -catenin occurs in a considerable proportion of gastric carcinomas, and there is a significant relationship between abnormal E-cadherin or β -catenin expression and tumor clinicopathology.

α -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^[5,6]. PC9 lung carcinoma cells, which do not have the α -catenin protein, could not bind tightly to each other despite the fact that they possess E-cadherin. Transfection of the α -catenin gene could restore their intercellular adhesion^[26]. As no methylation around the promoter region CpG island has been documented in the α -catenin gene^[4,36], gene deletions might be involved in reduced expression of α -catenin. In addition, post-transcriptional mechanisms may be important in the regulation of α -catenin function. E-cadherin might stabilize α -catenin protein through their specific binding to α -catenin^[37,38], or might increase the translation level of this molecule by decreasing the concentration of free α -catenin protein^[39]. In our study, we found loss of membranous α -catenin immunostaining in a subgroup of gastric carcinomas, and these results were in agreement with those of previous reports^[16,17,34,40]. However, these studies were based on immunohistochemical analyses and alteration of α -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 α -catenin was lost or downregulated, compared with the adjacent normal gastric mucosa or normal gastric tissues from healthy subjects. Of the 49 cases studied,

α -catenin expression was found to be downregulated or lost in 69% of gastric carcinomas. These results suggest that alteration of α -catenin occurs at the transcription level. We found that reduced α -catenin expression did not correlate with pT category or pN category, but correlated strongly with poor differentiation grade. Thus, our results suggest that α -catenin expression may be used as a differentiation marker. Moreover, our findings of reduced α -catenin mRNA expression in 5 of 26 tumor-free gastric mucosae suggest that changes in α -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*^[41], reported that *H pylori*-infected individuals exhibited downregulation of E-cadherin expression. Furthermore, Yu *et al*^[42], have previously shown that loss or downregulation of α -catenin mRNA in the gastric mucosa is associated with *H pylori* infection. However, in this study, there was no statistically significant correlation between α -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 α -catenin expression is common in gastric carcinoma, and may be an early event in gastric carcinoma. α -catenin expression may be used as a differentiation marker. Reduced α -catenin expression does not correlate with *H pylori* infection.

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