α-Catenin expression is decreased in human gastric cancers and in the gastric mucosa of first degree relatives

J Yu, M P A Ebert, S Miehlke, H Rost, U Lendeckel, A Leodolter, M Stolte, E Bayerdörffer, P Malfertheiner

Abstract

Background and aims—The role of altered cell adhesion is critical for the development of epithelial cancers. E-cadherin plays an important role in the maintenance of cell-cell adhesion and its function is thought to be regulated by its associated cytoplasmic proteins, such as α-catenin and β-catenin. To determine the role of α-catenin expression in gastric carcinogenesis, we studied its expression in human gastric cancer and in the gastric mucosa of first degree relatives with no clinical disease.

Methods—α-Catenin expression was assessed by immunohistochemical analysis and reverse transcriptase-polymerase chain reaction (RT-PCR) using gastric tissue specimens from patients with gastric cancer and from the gastric mucosa of first degree relatives of gastric cancer patients and healthy controls.

Results—mRNA levels of α-catenin were reduced or absent in 13 of 19 gastric cancer tissues, which differed significantly from levels found in the tumour free gastric mucosa of cancer patients (p<0.05). Of the cancer samples with altered α-catenin mRNA levels, α-catenin expression was negative in seven and decreased in six cases. Interestingly, decreased α-catenin mRNA expression also occurred in the mucosa of the corpus (11/18) and antrum (4/18) of first degree relatives. In the corpus biopsies α-catenin expression was more often decreased or lost compared with the antrum biopsies in first degree relatives and healthy controls (p<0.05). Immunohistochemical analysis revealed membranous expression of α-catenin in gastric cancer cells and the non-malignant gastric epithelium. However, some cancers also exhibited loss of membranous staining. Generally, loss or downregulation of α-catenin mRNA in the gastric mucosa was associated with *Helicobacter pylori* infection (p<0.05).

Conclusion—Our findings suggest that loss or downregulation of α-catenin expression may be an early event in gastric carcinogenesis and may be associated with *H pylori* infection.

Key words: risk; stomach; gene; cadherins; gastric cancer; α-catenin

Cadherins are calcium dependent transmembranous proteins which appear to mediate cell-cell adhesion through homophilic interactions. E-cadherin belongs to the cadherin superfamily and is involved in maintenance of the epithelial phenotype. Its function depends on the ability of its cytoplasmic sequence to link to the submembranous cytoskeletal matrix through interaction with proteins called catenins. Catenins have been classified into α, β, and γ based on electrophoretic mobility. They are necessary for E-cadherin functions, of which α-catenin is believed to be important in linking E-cadherin to the actin cytoskeleton and co-localises with E-cadherin at the adherens junction. The interaction of β-catenin bound to the cytoplasmic domain of E-cadherin with the actin cytoskeleton is mediated by α-catenin. Thus loss of α-catenin expression may lead to impaired formation of adherens junctions, despite normal E-cadherin and β-catenin expression.

Formation of metastases is a complex process which includes reduction of cell adhesion, invasion of neighbouring tissues, and release of cancer cells into the blood or lymphatic stream. However, loss of cell adhesion may not only contribute to formation of metastases, it may also be responsible for loss of contact inhibition of epithelial cells and thus may play a role in the early phase of malignant transformation of epithelial cells. Reduction or loss of E-cadherin expression has been described in gastric carcinoma, and mutations of E-cadherin occur in 50% of diffuse-type gastric carcinomas. Furthermore, reduced or total loss of α-catenin expression was observed in 55–70% of gastric cancers.

Therefore, alteration of expression of adhesion molecules seems to be a frequent event in gastric cancer. In tumours with normal E-cadherin expression, altered cell adhesion may also be explained by impaired function of catenins. Thus downregulation of α-catenin expression could also interfere with cadherin mediated cell-cell adhesion, leading to impaired adhesion in cases of normal E-cadherin expression in human cancerous tissue.

In this study we examined α-catenin expression in gastric cancer and in the gastric mucosa of first degree relatives of gastric cancer patients.
patients by RT-PCR and immunohistochemical analysis to assess the role of α-catenin in the process of gastric carcinogenesis and to evaluate its potential role as a marker for increased risk of developing cancer in patients with a family history of gastric cancer.

**Materials and methods**

**SUBJECTS**

Tissue specimens were obtained by surgical resection or biopsy from 19 patients with gastric cancer from the tumour and from a tumour free location. In all cases at least two biopsies were taken from the tumour and a tumour free area, which was located at least 6 cm distant from the tumour. Histological evaluation of one of the two biopsies confirmed the presence of tumour cells in the biopsy taken from the tumour and the absence of cancer cells in the biopsy taken from the non-tumour location. The other biopsy was used for molecular analysis and snap frozen in liquid nitrogen. Ten males and nine females (mean age 62 years (range 35–73)) were enrolled in the study. Tissues were also obtained from 18 first degree relatives of gastric cancer patients from the corpus and antrum (four males; mean age 47 years (range 27–65)). Five patients with gastric cancer and their five first degree relatives were enrolled in the study. Other patients and first degree relatives were from different families. Tissues from eight subjects with dyspepsia and normal gastric mucosa at endoscopy, but with no family history of gastric cancer and a history of peptic ulcers were used as normal controls. All individuals gave informed consent to participate in this study. Immediately after removal all tissues for molecular analysis were placed in liquid nitrogen and stored at −80°C until use. This study was approved by the ethics committees of the University of Magdeburg and the Technical University Hospital of Dresden, Germany.

**HISTOLOGY**

Formalin fixed tissues were processed as previously described and sections were stained with Haematoxylin and eosin for histological evaluation and with Warthin-Starry stain for detection of *Helicobacter pylori*.17 Gastric cancers were classified histologically as intestinal-type (n=9) or diffuse-type (n=9) according to the Lauren system. The severity of gastritis and *H pylori* colonisation of the non-tumorous gastric mucosa was histologically classified according to the updated Sydney system.17

**mRNA ISOLATION**

Tissue specimens were stored at −80°C and homogenised with an ultrasonic homogeniser (Ultra-Turrax T25, Janke and Kunkel GmbH, Germany) in the presence of RNazol B (CINNA/MRC, Cincinnati, Ohio, USA) containing RNase inhibitors. Total RNA was extracted from frozen tissues by the acid-guanidium-thiocyanate method as previously described.18

**RT-PCR ANALYSIS**

Total RNA (1 µg) was reverse transcribed using dNTPs (1 mM), DTT (5 mM), reverse transcription buffer (50 mM Tris HCl, pH 8.3, 75 mM KCl, 3 mM MgCl₂, and 1 mM DTT), and 40 U RNasin; 6 units of Superscript plus were added to a total volume of 20 µl. After incubation at 37°C for one hour the reaction was terminated by incubating the mixture at 95°C for 10 minutes. A pair of primers was designed to amplify the coding region of α-catenin mRNA. A sense primer, Pr-1 (5’-GAGCCAGTTTCTCAAGGAGGA-3’), corresponding to nucleotides 302–322, and an antisense primer, Pr-2 (5’-CGTGAGCTTCCTTAGGACGTC-3’), corresponding to nucleotides 691–711, were used.19 The 30 µl reaction mixture was incubated for 1.5 minutes at 95°C. Samples were denatured at 96°C for 24 seconds, annealed at 58°C for 48 seconds, and extended at 72°C for one minute. After 35 cycles there was a final elongation for five minutes at 72°C and the samples were stored at 4°C until further use; 8 µl of the PCR product was then electrophoresed on a 1.25% agarose gel together with size markers.

**SEMQUANTITATIVE ANALYSIS**

Levels of α-catenin mRNA were quantified using semiquantitative RT-PCR as previously described.20 β-Actin was used as an internal standard to confirm equal loading in each experiment and was amplified from the same cDNAs as previously described (fig 1).18 The level of α-catenin mRNA was analysed densitometrically from the agarose gel and was standardised to the respective β-actin mRNA level. The α-catenin/β-actin ratio was calculated and analysed using the t test.21

**IMMUNOHISTOCHEMISTRY**

The presence of α-catenin protein was assessed using formalin fixed, paraffin embedded gastric tissue sections obtained from gastric cancer patients (n=8), gastric cancer relatives (n=6), and healthy controls (n=6). Sections of 4 µm were deparaaffinised in graded alcohol.18 A highly specific polyclonal antibody for α-catenin (C-19) was obtained from Santa Cruz Biotechnology (Santa Cruz, California, USA). This antibody is directed against the carboxy terminus of α-catenin and has previously been shown to be specific for α-catenin.

![RT-PCR analysis of α-catenin mRNA in gastric tissues. RT-PCR analysis was performed using specific primers for human α-catenin as described in materials and methods. Amplification of β-actin mRNA was performed to ensure equal loading. Lanes 1–2: tumour free and tumour from patient No 1; lanes 3–4: corpus and antrum from relative No 6; lanes 5–7: tumour free and tumour from patient No 2; lanes 8–9: corpus and antrum from relative No 2; lanes 5 and 10: gastric mucosa from healthy subjects; lane 11: negative control; and lane 12: DNA ladder.](http://gut.bmj.com/)

**Figure 1**  RT-PCR analysis of α-catenin mRNA in gastric tissues. RT-PCR analysis was performed using specific primers for human α-catenin as described in materials and methods. Amplification of β-actin mRNA was performed to ensure equal loading. Lanes 1–2: tumour free and tumour from patient No 1; lanes 3–4: corpus and antrum from relative No 6; lanes 5–7: tumour free and tumour from patient No 2; lanes 8–9: corpus and antrum from relative No 2; lanes 5 and 10: gastric mucosa from healthy subjects; lane 11: negative control; and lane 12: DNA ladder.
α-Catenin expression in gastric cancer

using western blotting, immunoprecipitation, neutralisation experiments, and immunohistochemical analysis. For immunohistochemical analysis the antibody was used at a dilution of 1:100. For negative controls the primary antibody was omitted (not shown). All sections were subjected to microwave treatment for 2×7 minutes in citrate buffer. Endogenous peroxidase activity was inhibited by immersing the sections in 0.3% H2O2. The sections were incubated with the antibody at 4°C overnight. Bound antibody was detected with a biotinylated anti-goat IgG secondary antibody and streptavidin-peroxidase complex using diaminobenzidine tetrahydrochloride as the substrate. Sections were counterstained with Mayer’s haematoxylin.

STATISTICAL ANALYSIS

Fisher’s exact test, χ2 and t test were used to determine statistical differences, with p<0.05 taken as the level of significance.

Results

α-Catenin expression in gastric cancer

All gastric biopsies obtained from healthy control subjects exhibited α-catenin mRNA, as determined by RT-PCR analysis. In contrast, in 13 of 19 tumour samples, α-catenin mRNA levels were altered (table 1). Thus of the 19 gastric tumour tissues, expression of α-catenin was completely lost in seven (37%) and decreased in six (32%) compared with normal tissue (fig 1). In total, 13 (69%) tumour specimens exhibited low or absent α-catenin expression whereas in the tumour free locations reduced α-catenin expression or absence of α-catenin expression was observed in only four cases. Statistical analysis by χ2 test revealed that mRNA expression of α-catenin in the tumour location was significantly reduced compared with non-tumourous locations (p<0.05) (fig 2). Of the 19 gastric cancer specimens, nine were of the intestinal-type and nine of the diffuse-type. Seven of nine diffuse-type gastric cancers exhibited low α-catenin expression in addition to six of nine gastric cancers of the intestinal-type; thus there was no difference between α-catenin expression in the different histological types (table 1). Immunohistochemical analysis revealed membranous staining for α-catenin in non-malignant gastric epithelium of healthy controls (fig 3A). Immunoreactivity was abundant along the cell membranes (arrowheads) and cytoplasmic immunoreactivity was also observed in the non-malignant gastric mucosa (fig 3A). In gastric cancers, α-catenin expression was also membranous and cytoplasmic (fig 3B). However, some areas of the gastric cancers exhibited decreased α-catenin immunoreactivity (fig 3C). Expression of α-catenin in the tumour free gastric mucosa of gastric cancer patients was however not different from the membranous staining in the gastric mucosa of healthy individuals (not shown).

Table 1  α-Catenin expression in gastric cancer patients

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<tr>
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<th>Age</th>
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+, positive; ±, faint band; –, negative.
Hp, Helicobacter pylori.

α-catenin expression in the different histological types (table 1). Immunohistochemical analysis revealed membranous staining for α-catenin in non-malignant gastric epithelium of healthy controls (fig 3A). Immunoreactivity was abundant along the cell membranes (arrowheads) and cytoplasmic immunoreactivity was also observed in the non-malignant gastric mucosa (fig 3A). In gastric cancers, α-catenin expression was also membranous and cytoplasmic (fig 3B). However, some areas of the gastric cancers exhibited decreased α-catenin immunoreactivity (fig 3C). Expression of α-catenin in the tumour free gastric mucosa of gastric cancer patients was however not different from the membranous staining in the gastric mucosa of healthy individuals (not shown).

Expression of α-catenin in the gastric mucosa of first degree relatives

A total of 18 first degree relatives were examined for α-catenin mRNA expression using gastric tissues obtained from the corpus and antrum (table 2). In the biopsies obtained from the corpus, reduced levels of α-catenin expression were seen in six cases and no α-catenin expression in five (fig 1). In the antrum, decreased levels were observed in one case and loss of α-catenin expression was observed in three biopsies. Thus in the corpus biopsies α-catenin expression was more often decreased or lost than in the antrum biopsies in first degree relatives (p<0.05). In comparison with the gastric tissues from eight normal subjects, downregulation of α-catenin expression was found more frequently in tumour tissues (p<0.05) and in the corpus of relatives (p<0.05), but there was no significant variation between biopsies from normal subjects compared with non-tumourous tissues of cancer patients and antrum biopsies of first degree relatives (fig 2). Immunohistochemical analysis confirmed the membranous and cytoplasmic expression of α-catenin in the gastric mucosa of these high risk individuals. No difference in staining pattern was observed compared with healthy controls (fig 3A).

Correlation of α-catenin expression with H pylori infection

Of 35 tissue specimen obtained from the corpus and antrum of first degree relatives, the
Intercellular interactions are critical for the establishment and maintenance of normal cell morphology, differentiation, and growth control. Furthermore, disturbance or loss of normal cell-cell interactions as a result of defects in the function of adherens junctions may have a critical role in the malignant transformation of cells. Normal epithelial cells bind tightly to each other whereas cancer cells exhibit impaired adhesion.4,10 E-cadherin is a 120 kDa transmembrane glycoprotein that plays an important role in the organisation and maintenance of tissue structure by homotypic adhesion.1,2 E-cadherin is associated via its cytoplasmic domain with a group of catenins, including α-catenin, β-catenin, and γ-catenin/plakoglobin which act to couple the cadherins to the microfilament of the cytoskeleton.8 β-Catenin and γ-catenin/plakoglobin, both members of the armadillo protein family, link E-cadherin via α-catenin to the actin cytoskeleton.1,2,8 Recently, several studies have demonstrated that expression of E-cadherin and catenins is frequently downregulated in a number of human cancers.14,16,22 Furthermore, several groups have reported frequent mutations of the E-cadherin gene in various epithelial cancers.15,17,20 Thus it has been demonstrated by several groups that downregulation of E-cadherin expression and mutations of the E-cadherin gene occur in a subgroup of breast and gastric cancers.15,16,20 Interestingly, germ-line mutations of E-cadherin have also been reported in familial gastric cancer.25 In addition, an extensive study of E-cadherin and catenin expression in a large series of gastric cancers by immunohistochemical analysis has revealed a high frequency of abnormal expression of the E-cadherin–catenin complex in gastric cancers.14 In our study we also found loss of membranous α-catenin immunoreactivity in a subgroup of gastric cancer patients. However, our analysis of α-catenin mRNA levels revealed a further interesting difference between the normal gastric epithelium and gastric cancer cells. In our analysis α-catenin expression was present in the gastric mucosa of all healthy individuals whereas in the tumour tissues, gastritis was observed. Furthermore, the activity of gastritis was classified as low or moderate in 24 cases, whereas 10 cases exhibited no activity (table 2). No significant difference was found between the grade or activity of gastritis and the level of α-catenin expression. Interestingly, of the four biopsies with intestinal metaplasia, three cases exhibited low α-catenin expression (table 2). H pylori infection and mRNA expression of α-catenin was studied in 64 tissues from relatives and in the tumour free gastric mucosa of gastric cancer patients (table 3). Seventeen of 19 tissue specimens with H pylori infection exhibited reduced or no α-catenin expression, whereas in 22 tissue specimens with no H pylori infection only six exhibited low or no α-catenin expression. Statistical analysis by Fisher's exact test confirmed that reduced and/or no α-catenin expression was associated with H pylori infection (p<0.05) (table 3).

**Discussion**

Intercellular interactions are critical for the establishment and maintenance of normal cell morphology, differentiation, and growth control. Furthermore, disturbance or loss of normal cell-cell interactions as a result of defects in the function of adherens junctions may have a critical role in the malignant transformation of cells. Normal epithelial cells bind tightly to each other whereas cancer cells exhibit impaired adhesion.4,10 E-cadherin is a 120 kDa transmembrane glycoprotein that plays an important role in the organisation and maintenance of tissue structure by homotypic adhesion.1,2 E-cadherin is associated via its cytoplasmic domain with a group of catenins, including α-catenin, β-catenin, and γ-catenin/plakoglobin which act to couple the cadherins to the microfilament of the cytoskeleton.8 β-Catenin and γ-catenin/plakoglobin, both members of the armadillo protein family, link E-cadherin via α-catenin to the actin cytoskeleton.1,2,8 Recently, several studies have demonstrated that expression of E-cadherin and catenins is frequently downregulated in a number of human cancers.14,16,22 Furthermore, several groups have reported frequent mutations of the E-cadherin gene in various epithelial cancers.15,17,20 Thus it has been demonstrated by several groups that downregulation of E-cadherin expression and mutations of the E-cadherin gene occur in a subgroup of breast and gastric cancers.15,16,20 Interestingly, germ-line mutations of E-cadherin have also been reported in familial gastric cancer.25 In addition, an extensive study of E-cadherin and catenin expression in a large series of gastric cancers by immunohistochemical analysis has revealed a high frequency of abnormal expression of the E-cadherin–catenin complex in gastric cancers.14 In our study we also found loss of membranous α-catenin immunoreactivity in a subgroup of gastric cancer patients. However, our analysis of α-catenin mRNA levels revealed a further interesting difference between the normal gastric epithelium and gastric cancer cells. In our analysis α-catenin expression was present in the gastric mucosa of all healthy individuals whereas in the tumour tissues.
expression of α-catenin was lost or at least decreased in 69% of cases. The high frequency of decreased α-catenin mRNA levels in gastric cancers compared with normal gastric tissues obtained from healthy subjects highlights an important role of α-catenin in cell adhesion and maintenance of normal epithelial morphology in the healthy stomach, with the observed alteration in gastric cancer indicating decreased α-catenin expression in the development of gastric cancers. Our data are in line with previous reports which revealed altered expression of α-catenin in 50–70% of gastric cancers. However, these studies were based on immunohistochemical analyses and alteration of α-catenin expression was defined as abnormal non-membranous immunoreactivity in gastric cancer cells. Using a different approach, we demonstrated decreased levels of α-catenin mRNA in gastric cancer tissue specimens. In addition, our study revealed that α-catenin expression was abnormal in seven of nine diffuse-type cancers and in six of nine intestinal-type cancers. Thus although the number of cancer cases was small, we could not find a correlation between α-catenin expression and histological type of cancer according to the Lauren classification. This observation is compatible with data reported by Jawhari and colleagues. However, these authors revealed an association between abnormal expression of E-cadherin and γ-catenin and the diffuse-type gastric cancer.

The pathogenesis of gastric cancer remains largely unknown. Although a number of genetic and molecular alterations have been described in gastric carcinoma, the exact sequence and number of genetic or molecular alterations are not yet known. Apart from activation of oncogenes such as c-met and K-sam and inactivation of p53 and APC, alteration of adhesion molecules seems to be critical for the development of gastric cancers. Several models on how histomorphological changes of the gastric mucosa lead to the transformation of the gastric mucosa and the development of gastric cancer have been postulated. The best known was proposed by Correa et al who described the development of gastric tumours from gastritis to intestinal metaplasia, dysplasia, and eventually gastric cancer. Further studies have demonstrated that some histological changes, such as the presence of intestinal metaplasia or a corpus dominant gastritis in conjunction with intestinal metaplasia, define a group of individuals with an increased risk of developing gastric cancer.

Epidemiological analyses have shown that the incidence of gastric cancer in first degree relatives of patients with gastric cancer is increased threefold compared with the normal population. In addition, severe corpus mucosal atrophy has been reported in the stomach of these individuals and is associated with an increased risk of developing gastric cancer. Our analysis aimed at evaluating the role of α-catenin expression in the development of gastric cancer and its expression in the subgroup of these individuals with an increased risk of gastric cancer. Although immunohistochemical analysis did not reveal alteration of α-catenin protein expression in cancer relatives compared with healthy controls, we found that in the gastric mucosa of these individuals α-catenin mRNA levels in the corpus were downregulated compared with healthy controls. Moreover, expression of α-catenin in the corpus biopsies of these individuals was significantly lower than that in matched antrum biopsies. Although it seems as if α-catenin mRNA levels in the corpus biopsies from cancer relatives were lower compared with the non-tumour tissues of gastric cancer patients in our densitometric analysis, we believe that this reduction was caused by the heterogeneous sampling locations of non-tumour tissues in cancer patients and thus expression of α-catenin cannot be compared between these two groups. None the less, to our knowledge this is the first study reporting significant molecular changes of the gastric mucosa in this subgroup of individuals with an increased risk of gastric cancer. These observations support histomorphological findings which have determined an increased risk of cancer in siblings of gastric cancer patients who present with severe atrophy of the corpus mucosa. Furthermore, the presence of a corpus dominant gastritis together with intestinal metaplasia also contributes to an increased risk of gastric cancer. Our data extend these findings to the molecular level. α-Catenin mRNA levels were more often decreased in the corpus than in the antrum of individuals with an increased risk of developing gastric cancer and the mean level of expression in the corpus biopsies of these individuals was comparable with that in gastric cancer. In summary, our observation of decreased α-catenin expression in the corpus mucosa of first degree relatives raises the hypothesis that changes in the expression of α-catenin in the corpus mucosa in the presence of severe gastritis may represent early changes in the process of malignant transformation of the gastric mucosa.

Table 3  α-Catenin expression in tissues with (HP+) or without (HP−) H pylori infection

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<td>HP−</td>
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+, positive; −, faint band; −, negative.

α-Catenin was classified as a group I carcinogen by the International Agency for Research on Cancer in 1994 and several groups have reported that H pylori infection leads to enhanced cell proliferation and diminished apoptosis of the gastric mucosa, both...
features common in the process of malignant transformation. 1
9 Terres et al reported that H pylori infected individuals exhibit downregulation of E-cadherin expression in the antrum but no alteration of alpha 6-integrin or extracellular matrix protein expression (for example, collagen IV, fibronectin, or laminin) was reported. 36 We analysed the association of H pylori infection and alteration of alpha-catenin expression in a series of non-malignant gastric biopsies obtained from gastric cancer patients and high risk individuals and found an association between H pylori infection and decreased alpha-catenin expression. Thus together with previous reports revealing increased cell proliferation, diminished apoptosis, and downregulation of E-cadherin expression in the gastric mucosa of H pylori infected individuals, our findings of downregulation of alpha-catenin mRNA levels in this subgroup of individuals indicates the presence of multiple cellular and molecular alterations in the gastric mucosa of these individuals which may precede the development of gastric cancer.

In summary, we have shown that loss of alpha-catenin is common in gastric cancer, occurs in a proportion of first degree relatives, and is closely associated with H pylori infection. The presence of altered alpha-catenin expression in first degree relatives of patients with gastric cancer raises the hypothesis that molecular changes are present in the histologically tumour free gastric mucosa and may precede the development of gastric cancer. Further studies are necessary to assess the role of alpha-catenin mRNA levels for the identification of individuals with an increased risk of developing gastric cancer and their clinical management.

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