RESEARCH ARTICLE

Expression of the E-cadherin/β-catenin/tcf-4 Pathway in Gastric Diseases with Relation to *Helicobacter pylori* Infection: Clinical and Pathological Implications

Xiu-Wen Yu[1,2&], Qian Xu[1&], Ying Xu[1], Yue-Hua Gong[1], Yuan Yuan[1*]

Abstract

Objective: To determine the expression of E-cadherin, β-catenin, and transcription factor 4 (TCF4) proteins in gastric diseases with relation to *Helicobacter pylori* infection. **Methods:** A total of 309 patients including 60 with superficial gastritis (SG), 57 with atrophic gastritis (AG) and 192 with gastric cancer (GC), were enrolled. The expression of E-cadherin, β-catenin, TCF4 proteins in the gastric mucosa was detected by immunohistochemistry and *H. pylori* infection by immunohistochemistry and PCR. **Results:** The expression rates of E-cadherin were significantly higher in SG and AG than in GC (*P*<0.01), while those of β-catenin in the nucleus were significantly lower in SG and AG than in GC (*P*<0.05). In GC cases, the expression rates of E-cadherin, β-catenin and TCF4 were significantly higher in the intestinal type than in the diffuse type (*P*<0.05). In GC patients, the expression rate of E-cadherin was significantly higher in the presence of *H. pylori* than in the absence of infection (*P*=0.011). Moreover, the expression level of TCF4 and β-catenin protein was significantly higher in the nucleus and cytoplasm in *H. pylori* positive than in *H. pylori* negative GC patients, especially in those with the intestinal type (all *P* < 0.05). **Conclusion:** The expression of E-cadherin and β-catenin progressively decreases during the process of GC tumorigenesis, while overexpression of TCF4 occurs. *H. pylori* infection is associated with a significant increase in the expression of E-cadherin and β-catenin in the cytoplasm and nucleus in GC patients, especially those with the intestinal type.

Keywords: Gastric cancer - *H. pylori* - E-cadherin - β-catenin - transcription factor 4

Introduction

Gastric cancer (GC) is the most common gastrointestinal malignancy. The development of GC is a complex, multistage, and multifactorial process involving abnormal regulation of multiple genes (Kim et al., 2010). *Helicobacter pylori* infection is closely associated with the development of gastric carcinoma, and has been classified as a Group I carcinogen by the World Health Organization (WHO) (Watanabe et al., 2010). It has been reported that *H. pylori* infection induces GC by changing host gene and protein expression through several cell signaling pathways (Kim et al., 2010; Watanabe et al., 2010).

The Wnt pathway is confirmed to involve a large number of proteins that can regulate the production of Wnt signaling molecules, including Wnt proteins, frizzled, β-catenin and transcription factors (TCF). These molecules play important roles in cell differentiation, migration and proliferation. Among these molecules, β-catenin is the key factor in the Wnt pathway. Normally, β-catenin is integrated with E-Cadherin in the membrane, and they form a complex that mediates homocellular

adherence. However, aberrant expression of proteins in the complex has been reported in some epithelial cancers (Chelidonis et al., 2009; Pagaki et al., 2010). It is postulated that changes of the proteins in the complex may inhibit glycogen synthase kinase 3 (GSK-3)-mediated phosphorylation of β-catenin, allowing it to translocate from the membrane to the nucleus where it combines with TCF/LEF (Lymphoid enhancer-binding factor), interacts with transcription factors, regulates gene transcription, and consequently induces cell proliferation and even tumor formation (Bienz et al., 2000; Prasad et al., 2009; Wang et al., 2010b). Recently, E-Cadherin, Wnt, β-catenin and transcription factor 4 (TCF4) have been implicated in gastric carcinogenesis (Ding et al., 2010; Wu et al., 2010).

However, the effects of *H. pylori* infection on the expression of proteins in the Wnt/β-catenin pathway and the interactions between *H. pylori* infection and the Wnt/β-catenin pathway in gastric carcinogenesis have not been fully elucidated. Therefore, this study was carried out to determine the expression of E-cadherin, β-catenin, and TCF4 proteins in different stages of gastric carcinogenesis in relation to *H. pylori* infection.

[1]*Tumor Etiology and Screening Department of Cancer Institute and General Surgery, the First Affiliated Hospital of China Medical University, and Key Laboratory of Cancer Etiology and Prevention (China Medical University), Liaoning Provincial Education Department, Shenyang,* [2]*Department of Pathology, Qiqihar Medical College, Qiqihar, Heilongjiang, China* [&]*Equal contributors* *For correspondence: yyuan@mail.cmu.edu.cn*

Materials and Methods

Patients and Study design

The study protocol was approved by the Ethics Committee of China Medical University. A total of 309 patients (including 194 males and 115 females, with mean age 59.15 ranged from 30-79), from 1992 to 2007 participating in a health check program for gastric cancer screening in Zhuanghe of Liaoning Province, China, were included in the study, where the gastric cancer screening were done by China Medical University. They were histologically diagnosed with superficial gastritis (SG) (n=60), atrophic gastritis (AG) (n=57) and gastric cancer (GC) (n=192). Classification and grading of gastritis was based on Updated Sydney System (Dixon MF, et al., 1996; Stolte M, et al., 2001) . According to Lauren's classification (Lauren, 1965), 97 of the 192 GC cases were the intestinal type and 95 were the diffuse type. Gastric specimens that were taken from gastric sites with apparent lesions or from resected gastric tumors were fixed in 95% ethanol, embedded in paraffin, and cut into 5 μm slices. These specimens were used for histological examinations with hematoxylin and eosin staining as described above, and for immunohistochemical assessment of the proteins as describe below.

Immunohistochemistry for detection of E-cadherin, β-catenin and TCF4 protein expression

Immunohistochemical methods were adopted for the expression of E-cadherin, β-catenin and TCF4 proteins. The primary antibodies were rabbit anti-human E-cadherin (H-108, dilution 1:100, Santa Cruz, California, USA), β-catenin (E5, dilution 1:200, Santa Cruz), and TCF4 (EP2033Y, dilution 1:200 Epitomics, Burlingame, USA) monoclonal IgG antibodies. The amplification system that was used in the reaction was the streptavidin-peroxidase kit (Maixin Biotechnology Co. Ltd, Fujian, China). Sections were dewaxed, and incubated with methanol containing 3% H_2O_2 for 20 min to block endogenous peroxidase activity. To enhance antigen retrieval, sections were treated in a microwave oven. Briefly, sections were immersed in 0.01 mol/L citrate buffer (pH 6.0) and heated in a microwave oven at 100°C for 20 min. Subsequently, they were washed three times with distilled water, and then blocked with 1% bovine serum albumin for 30 min. Sections were incubated overnight at 4°C with rabbit polyclonal IgG of *H. pylori*, E-cadherin, β-catenin and TCF4. A subsequent reaction was carried out using second antibodies (Maixin Biotechnology Co. Ltd, Fujian, China) for 15 min at 37°C. Sections were washed three times with phosphate buffered saline (PBS) buffer and subsequently displayed color with the usage of diaminobenzidine (Zhongshan Goldenbridge Biotechnology Co. Ltd, Beijing, China) for about 5 min. Nuclei were lightly counterstained with hematoxylin. Immunohistochemical staining for E-cadherin, β-catenin and TCF4 in the *H. pylori* negative GC tissue was used as a positive control for the expression of these proteins. Staining using PBS, instead of the primary antibody, was used as the negative control.

A comprehensive assessment (overall scoring) system (Yu et al., 2009) was used to determine the expression of E-Cadherin staining in the cell membrane, TCF4 in the nucleus, and β-catenin in the cell membrane, cytoplasm and nucleus. Briefly, five visual fields were randomly selected under a light microscope (×400) to assess the number of positively immunostained cells in a total of 100 cells per visual field and the intensity of immunostaining. Then, a comprehensive assessment was performed according to the ratio of positive cells and staining intensity (Yu et al., 2009). The positive cell ratio score was classified semi-quantitatively according to the following criteria: 0, < 5% of cells discretely expressed; 1, ≥ 5 and < 25% of cells discretely expressed; 2, ≥26% and <50% of cells discretely expressed; and 3, ≥ 50% of cells discretely expressed. The staining intensity score was classified according to the following criteria: 0, no brown granules in the cells; 1, light brown granules in the cells; 2, obvious brown granules in the cells; and 3, strong brown or brown-yellow granules in the cells. The overall score was calculated by the positive cell ratio score × the staining intensity score. A patient with the final score of 1 or more was defined as positive for the expression of the protein.

Detection of H. pylori infection

H. pylori infection was detected by immunohisto-chemistry and polymerase chain reaction (PCR) method.

The immunohistochemistry was carryout as described as above, except that the primary antibody was *H. pylori*-IgG (DAKO, Denmark A/S, 1:25), and the secondary antibody was rabbit polyclonal IgG of *H. pylori*. In addition, immunohistochemical staining of a smear of cultured *H. pylori* bacteria was used as a positive control for the presence of *H. pylori* infection. The presence of *H. pylori* bacterial cells were observed on the surface of gastric epithelium, in the gastric pits or lumens of the glands within the gastric mucosa. *H. pylori* infection was considered positive if yellow or brownish yellow stained short rod or curved shape bacilli in immunohistochemistry staining sections were observed. The presence and the density of *H. pylori* infection were determined by the mean number of immunostained bacteria of two visual fields under the light microscope (×1000) (Liu et al., 2008): 0, no bacteria; 1, ≥ 1 and <9 bacteria; 2, ≥10 and <29 bacteria; 3, ≥30 and <99 bacteria; and 4, ≥100 bacteria. A specimen with a density score of 1 or more was defined positive for *H. pylori*.

For PCR, DNA samples were extracted from the paraffin fixed gastric specimens using WaxFREETMDNA Kit (Quick DNA preparation for FFEP; TrimGen, Cat. No. WF-100). *H. pylori* 16s rRNA and glmM (formally ureC) genes were detected using polymerase chain reaction (PCR) method, as previously described (Lu et al., 1999; Riggio et al., 2000). The primer sequences for PCR were as follows: 16s rRNA, forward primer, 5'-CGTTAGCTGCATTACTGGAGA-3', and reverse primer 5'-GAGCGCGTAGGCGGGATAGTC-3'; glmM, forward primer, 5'-AAGCTTTTAGGGGGTGTTAGGGGT TT-3', and reverse primer 5'-AAGCTTACTTTCTAACAC TAACGC-3'. The expected amplification products were 295 bp and 294bp, respectively. PCR cycling conditions

Table 1. Expression of E-Cadherin, B-Catenin and TCF4 Proteins in the Normal Gastric Mucosa, Superficial Gastritis (SG), Atrophic Gastritis (AG) and Gastric Cancer (GC)

Groups	E-cadherin (n (%))	β-catenin (n (%))	TCF4 (n (%))
SG (n=60)	60 (100)*	47 (78.3)	48 (80.0)
AG (n=57)	57 (100)*	38 (66.7)	48 (84.2)
GC (n=192)	156 (81.3)	130 (67.7)	166 (86.5)
Intestinal type (n=97)	93 (95.9)**	79 (81.4)**	91 (93.8)***
Diffuse type (n=95)	63 (66.3)	51 (53.7)	75 (78.9)

*P<0.01, compared with GC; **P<0.001, compared with the diffuse type; ***P=0.003, compared with the diffuse type

Table 2. Expression of B-Catenin Protein in Different Cellular Localization of the Normal Gastric Mucosa, Superficial Gastritis (SG), Atrophic Gastritis (AG) and Gastric Cancer (GC)

Groups	n	Membrane n (%)	Cytoplasm n (%)	Nucleus n (%)
SG	60	47 (78.3) ******	34 (56.7)	2 (3.3) *
AG	57	38 (66.7)	30 (52.6)	2 (3.5) **
GC	192	122 (63.5)	101 (52.6)	29 (15.1)
Intestinal type	97	75 (77.3) ***	66 (68.0) ****	23 (23.7) *****
Diffuse type	95	47 (49.5)	35 (36.8)	6 (6.3)

Compared to GC, *P=0.015, **P=0.020, ******P=0.033; Compared to the diffuse type, ***P=0.000, ****P=0.000, *****P=0.001

Figure 1. Expression of E-cadherin, β-catenin and TCF4 Proteins in Superficial Gastritis (SG), Atrophic Gastritis (AG) and Gastric Cancer (GC) (×200). A, E-cadherin expression in SG (A1), AG (A2), intestinal type of GC (A3) and diffuse type of GC (A4). B, β-catenin expression in SG (B1, mainly in the membrane), AG (B2, mainly in the membrane), intestinal type of GC (B3, mainly in the cytoplasm and nucleus) and diffuse type of GC (B4, mainly in the cytoplasm and nucleus). C, TCF4 expression in the SG (C1), AG (C2), intestinal type of GC (C3) and diffuse type of GC (C4)

consisted of initial annealing at 94 °C for 5 min, 35 cycles of reaction at 94 °C for 45sec, 55 °C for 45sec and 72 °C for 45sec and final extension at 72 °C for 7 min. PCR products were then separated by electrophoresis on a 2% agarose gel stained with 5% ethidium bromide, and further confirmed by BDT Sequencing (ABI377 DNA Sequencer, Applied Biosystems, USA). A specimen was considered to be positive for *H. pylori* if the two genes were detected. Finally, a patient was defined to be positive for *H. pylori* infection if the specimen was positive by any two of the three used methods.

Statistical analysis

Statistics analysis was performed using the SPSS11.5 statistical software package (SPSS Inc. Chicago, USA). Intergroup comparison was conducted using the χ^2 test or Fisher's exact test. All statistical tests were bilateral probability tests. A *P* value of < 0.05 was considered statistically significant.

Results

The expression of E-cadherin, β-catenin and TCF4 proteins in patients with SG, AG and GC

E-cadherin protein expression was located in the cell membrane (Figures 1A). The positive rate of E-cadherin protein expression was significantly higher in SG (100%) and AG (100%) than in GC (81.3%, P<0.01, respectively), while there was no significant difference between SG and AG. In GC patients, E-cadherin protein expression rate was higher in the intestinal type GC than in the diffuse type GC (95.9% vs. 66.3%, P<0.001, Table 1).

β-catenin protein expression was also located in the cell membrane, cytoplasm and/or nucleus (Figures 1B). The positive rate of β-catenin expression was higher, albeit not statistically significantly, in SG (78.3%) than
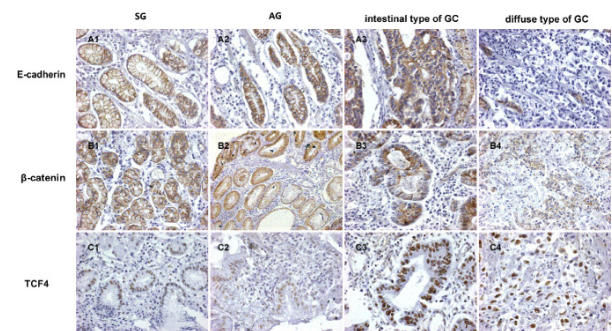
in AG (66.7%) and GC (67.7%, Table 1), regardless of expression location (SG vs. AG, χ^2=2.002, P=0.157 and SG vs. GC, χ^2=2.469, P=0.116.). In the cell membrane, the positive rate of β-catenin expression was higher in SG (78.3%) than in AG (66.7%), and GC (63.5%) (SG vs. AG, χ^2=2.002, P=0.157 and SG vs. GC, χ^2=4.528, P=0.033). In the cell cytoplasm, the positive rate of β-catenin protein expression was decreased gradually from SG to AG and then to GC, (56.7%, 52.6% and 52.6%, respectively) (SG vs. AG, χ^2=0.192, P=0.661; SG vs. GC, χ^2=0.303, P=0.582). On the other hand, in the nucleus, β-catenin protein was more frequently expressed in GC (15.1%) than in SG (3.3%) and AG (3.5%) (GC vs. SG, χ^2=5.871, P=0.015, and GC vs. AG, χ^2=5.422, P=0.020). Moreover, in GC cases, the positive rate of β-catenin protein expression was significantly higher in the intestinal type than in the diffuse type in the cell membrane (77.3% vs. 49.5%, χ^2=16.064, P=0.000), cytoplasm (68.0% vs. 36.8%, χ^2=18.738, P=0.000) or nucleus (23.7% vs. 6.3%, χ^2=11.326, P=0.001) (Tables 1 & 2).

TCF4 protein expressed in the nucleus (Figures 1C), and there was no significant difference in the expression rate of the protein among SG, AG and GC. However, in GC cases, the positive rate of TCF4 protein expression was higher in the intestinal type than in the diffuse type (93.8% vs. 78.9%, χ^2=9.061, P=0.003) (Table 1).

Association between H. pylori infection and the expression of E-cadherin, β-catenin and TCF4 proteins in patients with SG, AG and GC

Under the high-magnification microscope, *H. pylori* bacterial cells were presented in distant mucosal surface mucus, on the surface epithelium of gastric pits, or in the glandular cavity in many cases with SG, AG and GC (Figure 2). Detection of *H. pylori* 16s rRNA and glmM genes is shown in Figure 3. Overall .*H. pylori* infection was present in SG (85.0%, 51/60), AG (87.7%, 50/57) and GC (60.4%, 116/192) of patients; the rates were significantly higher in SG and AG than in GC (SG vs. GC χ^2=12.359, P=0.000; and AG vs. GC χ^2=14.743, P=0.000,

Table 3. Association Between *H. pylori(Hp)* Infection and the Expression of E-Cadherin, B-Catenin and Tcf4 Proteins in Superficial Gastritis(SG), Atrophic Gastritis(AG) and Gastric Cancer(GC)

	Superficial gastritis		Atrophic gastritis		Gastric cancer	
	Hp negative	*Hp* positive	*Hp* negative	*Hp* positive	*Hp* negative	*Hp* positive
n	9	51	7	50	76	116
E-cadherin	9(100.0)	51(100.0)	7(100.0)	50(100.0)	55(72.4)	101(87.1)*
β-catenin	9(100.0)	38(74.5)	6(85.7)	32(64.0)	52(66.5)	78(73.5)
Membrane	9(100.0)	38(74.5)	6(85.7)	32(64.0)	47(61.8)	75(64.7)
Cytoplasm	6(66.7)	28(54.9)	4(57.1)	26(52.0)	32(42.1)	69(68.3)**
Nucleus	0(0.0)	2(2.9)	0(0.0)	2(4.0)	6(7.9)	23(19.8)***
TCF4	8(88.9)	40(78.4)	6(85.7)	42(84.0)	62(81.6)	104(89.7)

Compared to the *H. pylori* negative group: *$P=0.011$; **$P=0.018$; and ***$P=0.024$

Table 4. Association Between *H. pylori (Hp)* Infection and E-Cadherin, B-Catenin and Tcf4 Protein Expression in Different Histological Type of Gc

	Intestinal type		Diffuse type	
	Hp negative	*Hp* positive	*Hp* negative	*Hp* positive
n	31	66	45	50
E-cadherin	29(93.5)	64 (97.0)	26 (57.8)	37 (74.0)
β-catenin	24 (77.4)	55(83.3)	28 (62.2)	23 (46.0)
Membrane	25 (80.6)	50(75.8)	22 (48.9)	25 (50.0)
Cytoplasm	14 (45.2)	52 (78.8)*	18 (40.0)	17(34.0)
Nucleus	3 (9.7)	20(30.0)**	3(6.7)	3 (6.0)
TCF4	26 (83.9)	65 (98.5)***	36 (80.0)	39 (78.0)

compared with *Hp* negative group: *$P=0.001$, and **$P=0.026$, ***$P=0.012$

Figure 2. *H. pylori* **Infection as Detected by Immunohistochemistry in SG (A) and GC (B).** Arrows point to the bacteria on the on the surface epithelium of gastric pits (A), or in the glandular cavity (B)

Figure 3. Representatives of Polymerase Chain Reaction for the Detection of *H. pylori* 16S Rrna and Glmm (Formally Urec) Genes in Superficial Gastritis (Sg, Lanes 1, 2, and 3), Atrophic Gastritis (Ag, Lanes 4, 5, and 6) and Gastric Cancer (Gc, Lanes 7, 8, and 9)

respectively). In GC patients, a significant difference was found in the *H. pylori* detection rate between intestinal the type and diffuse type GC (68.0% vs. 52.6%, $\chi^2=4.766$, $P=0.029$).

In SG and AG groups, no significant difference in the expression of E-cadherin, β-catenin and TCF4 proteins was observed between *H. pylori* negative and positive cases. There was also no significant difference in β-catenin protein expression between *H. pylori* negative and positive cases in terms of location. However, in the GC group, the positive rate of E-cadherin protein expression was higher in *H. pylori* positive cases than in *H. pylori* negative cases (87.1% vs. 72.4%, $\chi^2=6.513$, $P=0.011$). On the other hand, although the overall positive rate of β-catenin and TCF4 protein expression was not significantly different, the positive rate of β-catenin protein expression was higher in both the cytoplasm (68.3% vs. 42.1%, $\chi^2=5.561$, $P=0.018$) and nucleus (19.8% vs. 7.9%, $\chi^2=5.099$, $P=0.024$) in *H. pylori* positive cases compared with *H. pylori* negative cases (Table 3).

In intestinal type GC cases, no significant difference was observed in E-cadherin and β-catenin protein expression between *H. pylori* negative and *H. pylori* positive cases. However, the positive rate of β-catenin protein expression was higher in both the cytoplasm (78.8% vs. 45.2%, $\chi^2=10.968$, $P=0.001$) and nucleus (30.0% vs. 9.7%, $\chi^2=5.297$, $P=0.021$) in *H. pylori* positive cases compared with *H. pylori* negative cases. TCF4 protein was significantly higher expressed in *H. pylori* positive cases compared with those with negative *H. pylori* infection (98.5% vs. 83.9%, $\chi^2=7.763$, $P=0.012$).In the diffuse type cases, no significant difference was observed
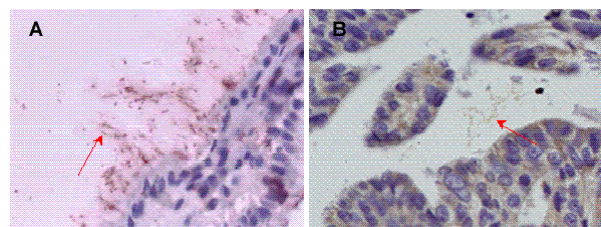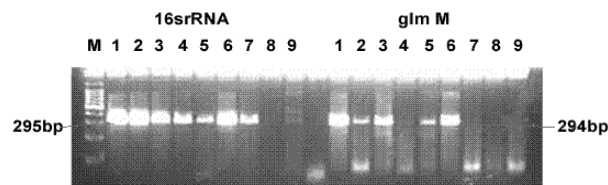
in E-cadherin, β-catenin and TCF4 protein expression between *H. pylori* negative and *H. pylori* positive cases, either overall or in terms of the locations (Table 4).

Discussion

The development and progression of GC involves a number of genetic and epigenetic abnormalities. At present, although the roles of *H. pylori* infection and Wnt/β-catenin in the development and metastasis of GC have been independently studied (Macleod RJ, 2012; Neal JT, et al., 2013), the association between *H. pylori* infection and Wnt/β-catenin in the development and progression of gastric carcinoma remains to be clarified (Ilyas, 2005; McMillan et al., 2005). Thus, it is essential to simultaneously examine *H. pylori* infection and the expression of E-cadherin, β-catenin and TCF4 proteins, and their association in various gastric mucosal lesions, thereby providing an additional theoretical basis on GC tumorigenesis and progression.

E-cadherin is a calcium-dependent cell adhesion molecule binding of β-catenin in normal epithelial cells (Aoki et al., 2007). When E-cadherin expression declines, the cell adhesion among tumor cells in the primary tumor is weakened; hence, tumor cells invasion and metastasis would easily occur. In our study, we found that E-cadherin expression was decreased in the progress from gastritis to GC, and the intestinal type of GC had a higher expression compared with the diffuse type of GC. Tamura et al. reported that E-cadherin expression was decreased in diffuse GC due to methylation, and that decreased expression of E-cadherin reduced the content of combined β-catenin in the complex (Tamura, 2008). β-catenin is a multifunctional protein found in three cell compartments: the plasma membrane, the cytoplasm and the nucleus. The cell has developed elaborate ways of regulating the level and localization of β-catenin to

assure its specific function in each compartment. In the normal tissue, the Wnt signaling pathway is turned off, and β-catenin mainly locates in the cell membrane at a low level. However, either β-catenin expression increases or ectopic expression of β-catenin occurs in the malignant tumors (Ogasawara N, et al., 2006; *P*andurangan AK, 2013). In our study, β-catenin membrane expression was decreased following the progression from SG to GC, while the expression in the nucleus was increased. In addition, the positive rate of β-catenin expression in the intestinal type of GC was significantly higher than that in the diffuse type of GC, regardless of the location (i.e. the membrane, cytoplasm and nucleus). These finding suggests that ectopic expression of β-catenin is more closely related to the intestinal type of GC than the diffuse type of GC. There was a report based on the similar topic (Ogasawara N, et al., 2006). In a retrospective study of 157 gastric carcinomas using immunohistochemistry and molecular genetics, Nabais et al. concluded that the pattern of β-catenin expression is closely related to the histological types of gastric cancer (Nabais et al., 2003).

TCF4 is the downstream protein of the Wnt/β-catenin signaling pathway and plays the role of a molecular switch in the pathway (Thevenod et al., 2010). When the Wnt/β-catenin signaling pathway is activated, β-catenin protein competitively binds TCF after entering the nucleus; thereby the transcription of target genes is enhanced (Thevenod et al., 2010). In the present study, TCF4 protein expression was increased with the aggravation of gastric mucosal lesions, and the positive rate of the expression in intestinal type GC was higher than that in diffuse GC. Following the aggravation of gastric mucosal lesions, E-cadherin expression was reduced, resulting in the ectopic expression of β-catenin protein in the nucleus, where β-catenin acts as a TCF binding factor and an activator for transcription by the displacement of Groucho-HDAC co-repressors (Kavak et al., 2010; Wang et al., 2010a).

H. pylori infection is closely associated with the development of gastric carcinoma, but the specific mechanism is still unclear (Ding et al., 2010; Osman et al., 2013). It has been shown that *H. pylori* induces cell proliferation through MA*P*K and NF-kappaB, thereby contributing to GC (Sibony et al., 2012). On the other hand, it is acknowledged that the Wnt/β-catenin signaling pathway is the most important pathway in the process of gastrointestinal tract tumor tumorigenesis and development. Therefore, a question of whether or not there was an association between *H. pylori* infection and the Wnt/β-catenin signaling pathway in GC tumorigenesis was raised. Kirikoshi et al. suggested that up-regulation of WNT10A induced by TNF-alpha and *H. pylori* infection might play key roles in human GC through activation of the Wnt/β-catenin signaling pathway (Kirikoshi et al., 2001). The sequence of events in *H. pylori*-associated gastric carcinogenesis is probably as follows: normal gastric mucosa, SG, AG, IM, dysplasia and GC (Correa et al., 2007; Correa et al., 2012). In our study, the rate of *H. pylori* infection was lower in GC cases (60.4%), compared with those in SG (85.0%) and AG (87.7%)

cases. Our findings were similar to those obtained in a Chinese study which showed that whereas the rates were 63.3% and 80.8%, respectively, in gastric erosion and gastric ulcer, the overall rate was 52.4% in patients with early gastric cancer, with the rates being 35.0%, 50.7%, 34.6%, respectively, in the antrum, corpus, and incisura of stomach (Zhang et al., 2005). Indeed, it has been shown that development of precancerous and cancerous lesions may create a hostile environment to *H. pylori*, and thus *H. pylori* infection may disappear following the development of these lesions (Genta et al., 1993). In GC cases, the E-cadherin expression level was higher in *H. pylori* positive than in *H. pylori* negative cases, and the β-catenin protein expression level in the cytoplasm and nucleus was higher in *H. pylori* positive than in *H. pylori* negative cases. Kurashima et al. reported that infection with *H. pylori* cagA-positive strains caused gastritis and peptic ulceration, and was associated with gastric adenocarcinoma (Kurashima et al., 2008). CagA also destabilizes the E-cadherin/β-catenin complex to elicit aberrant activation of the β-catenin signal that underlies intestinal metaplasia (Correa et al., 2007; Correa et al., 2011; Correa et al., 2012).

At present, most researchers believe that *H. pylori* and the Wnt/β-catenin signaling pathway play an important part in the intestinal type of GC tumorigenesis (Jin et al., 2008; Tuynman et al., 2008). However, the correlation between *H. pylori* infection and the Wnt/β-catenin signaling pathway in GC tumorigenesis is unclear. The present study showed that β-catenin protein expression level in the cytoplasm and the nucleus was higher in *H. pylori* positive than in *H. pylori* negative patients with GC, especially those with the intestinal type, in addition the similar phenomenon was also observed in TCF4 protein, which indicates that *H. pylori* infection can cause an E-cadherin/β-catenin complex fracture, so that β-catenin could enter the nucleus and bind to TCF4, resulting in increased TCF4 protein expression, which then subsequently contributes to the occurrence of intestinal-type GC.

In conclusion, the expression of E-cadherin and β-catenin is progressively decreased following the process of GC tumorigenesis, with β-catenin expression being translocated from the cytoplasm to the nucleus, contrarily, overexpression of TCF4 was observed in GC carcinogenesis process. *H. pylori* infection is associated with a significant increase in the expression of E-Cadherin and β-catenin in the cytoplasm and nucleus in patients with gastric cancer. In intestinal-type GC group, higher TCF4 and β-catenin in the cytoplasm and nucleus present in *H. pylori* infection cases.

Acknowledgements

References

Aoki R, Yasuda M, Torisu R, et al (2007). Relationship between lymph node metastasis and E-cadherin expression in submucosal invasive gastric carcinomas with gastric-phenotype. *J Med Invest*, **54**, 159-67.

Bienz M, Clevers H (2000). Linking colorectal cancer to Wnt signaling. *Cell*, **103**, 311-20.

Chelidonis G, Kavantzas N, Patsouris E, et al (2009). DNA ploidy, E-cadherin, beta-catenin expression and their clinicopathologic significance in imprints of non-small cell lung cancer. *Anal Quant Cytol Histol*, **31**, 332-9.

Correa P, Houghton J (2007). Carcinogenesis of *Helicobacter pylori*. *Gastroenterology*, **133**, 659-72.

Correa P, Piazuelo MB (2011). *Helicobacter pylori* infection and gastric adenocarcinoma. *US Gastroenterol Hepatol Rev*, **7**, 59-64.

Correa P, Piazuelo MB (2012). Evolutionary History of the *Helicobacter pylori* Genome: Implications for Gastric Carcinogenesis. *Gut Liver*, **6**, 21-8.

Ding SZ, Goldberg JB, Hatakeyama M (2010). *Helicobacter pylori* infection, oncogenic pathways and epigenetic mechanisms in gastric carcinogenesis. *Future Oncol*, **6**, 851-62.

Dixon MF, Genta RM, Yardley JH, Correa P (1996). Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol*, **20**, 1161-81.

Genta RM, Graham DY (1993). Intestinal metaplasia, not atrophy or achlorhydria, creates a hostile environment for Helicobacter pylori. *Scand J Gastroenterol*, **28**, 924-8.

Ilyas M (2005). Wnt signalling and the mechanistic basis of tumour development. *J Pathol*, **205**, 130-44.

Jin T, George Fantus I, Sun J (2008). Wnt and beyond Wnt: multiple mechanisms control the transcriptional property of beta-catenin. *Cell Signal*, **20**, 1697-704.

Kavak E, Najafov A, Ozturk N, et al (2010). Analysis of the Wnt/B-catenin/TCF4 pathway using SAGE, genome-wide microarray and promoter analysis: Identification of BRI3 and HSF2 as novel targets. *Cell Signal*, **22**, 1523-35.

Kim JH, Choi YJ, Lee SH, et al (2010). Effect of *Helicobacter pylori* infection on the sonic hedgehog signaling pathway in gastric cancer cells. *Oncol Rep*, **23**, 1523-8.

Kirikoshi H, Sekihara H, Katoh M (2001). Up-regulation of WNT10A by tumor necrosis factor alpha and *Helicobacter pylori* in gastric cancer. *Int J Oncol*, **19**, 533-6.

Kurashima Y, Murata-Kamiya N, Kikuchi K, et al (2008). Deregulation of beta-catenin signal by *Helicobacter pylori* CagA requires the CagA-multimerization sequence. *Int J Cancer*, **122**, 823-31.

Lauren P (1965). The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma - an attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand*, **64**, 31-49.

Liu YE, Yu XW, Dong NN, et al (2008). The conditions of *Helicobacter pylori* colonization in different gastric diseases. *Zhonghua Yi Xue Za Zhi*, **88**, 230-2 (in Chinese).

Lu JJ, Perng CL, Shyu RY, et al (1999). Comparison of five PCR methods for detection of *Helicobacter pylori* DNA in gastric tissues. *J Clin Microbiol*, **37**, 772-4.

Macleod RJ (2012). MDR-1, Bcl-xL, *H. pylori*, and Wnt/beta-catenin signalling in the adult stomach: how much is too much? *Lab Invest*, **92**, 1670-3.

McMillan M, Kahn M (2005). Investigating Wnt signaling: a chemogenomic safari. *Drug Discov Today*, **10**, 1467-74.

Nabais S, Machado JC, Lopes C, et al (2003). Patterns of beta-catenin expression in gastric carcinoma: clinicopathological relevance and mutation analysis. *Int J Surg Pathol*, **11**, 1-9.

Neal JT, Peterson TS, Kent ML, Guillemin K (2013). *H. pylori* virulence factor CagA increases intestinal cell proliferation by Wnt pathway activation in a transgenic zebrafish model. *Dis Model Mech*, **6**, 802-10.

Ogasawara N, Tsukamoto T, Mizoshita T, et al (2006). Mutations and nuclear accumulation of beta-catenin correlate with intestinal phenotypic expression in human gastric cancer. *Histopathology*, **49**, 612-21.

Osman MA, Bloom GS, Tagoe EA (2013). *Helicobacter pylori*-induced alteration of epithelial cell signaling and polarity: a possible mechanism of gastric carcinoma etiology and disparity. *Cytoskeleton (Hoboken)*, **70**, 349-59.

Pagaki E, Patsouris E, Gonidi M, et al (2010). The value of E-cadherin/beta-catenin expression in imprints of NCSLC: relationship with clinicopathological factors. *Diagn Cytopathol*, **38**, 419-24.

Pandurangan AK (2013). Potential targets for prevention of colorectal cancer: a focus on PI3K/Akt/mTOR and Wnt pathways. *Asian Pac J Cancer Prev*, **14**, 2201-5.

Prasad CP, Rath G, Mathur S, et al (2009). Expression analysis of E-cadherin, Slug and GSK3beta in invasive ductal carcinoma of breast. *BMC Cancer*, **9**, 325.

Riggio MP, Lennon A, Wray D (2000). Detection of *Helicobacter pylori* DNA in recurrent aphthous stomatitis tissue by PCR. *J Oral Pathol Med*, **29**, 507-13.

Sibony M, Jones NL (2012). Recent advances in *Helicobacter pylori* pathogenesis. *Curr Opin Gastroenterol*, **28**, 30-5.

Stolte M, Meining A (2001). The updated Sydney system: classification and grading of gastritis as the basis of diagnosis and treatment. *Can J Gastroenterol*, **15**, 591-8.

Tamura G (2008). Gastric cancer: histological type, histogenesis, and gene abnormalities. *Gan To Kagaku Ryoho*, **35**, 343-9 (in Japanese).

Thevenod F, Chakraborty PK (2010). The role of Wnt/beta-catenin signaling in renal carcinogenesis: lessons from cadmium toxicity studies. *Curr Mol Med*, **10**, 387-404.

Tuynman JB, Vermeulen L, Boon EM, et al (2008). Cyclooxygenase-2 inhibition inhibits c-Met kinase activity and Wnt activity in colon cancer. *Cancer Res*, **68**, 1213-20.

Wang Q, Sun ZX, Allgayer H, Yang HS (2010a). Downregulation of E-cadherin is an essential event in activating beta-catenin/Tcf-dependent transcription and expression of its target genes in Pdcd4 knockdown cells. *Oncogene*, **29**, 128-38.

Wang Q, Symes AJ, Kane CA, et al (2010b). A novel role for Wnt/Ca2+ signaling in actin cytoskeleton remodeling and cell motility in prostate cancer. *PloS One*, **5**, e10456.

Watanabe T, Asano N, Fichtner-Feigl S, et al (2010). NOD1 contributes to mouse host defense against *Helicobacter pylori* via induction of type I IFN and activation of the ISGF3 signaling pathway. *J Clin Invest*, **120**, 1645-62.

Wu WK, Cho CH, Lee CW, et al (2010). Dysregulation of cellular signaling in gastric cancer. *Cancer Lett*, **295**, 144-53.

Yu XW, Xu Q, Gong YH, et al (2009). Detection *in situ* of E-cadherin, β-catenin, TCF4 and CDX2 in various gastric diseases. *Chin J Cancer Res*, **21**, 185-93.

Zhang C, Yamada N, Wu YL, et al (2005). *Helicobacter pylori* infection, glandular atrophy and intestinal metaplasia in superficial gastritis, gastric erosion, erosive gastritis, gastric ulcer and early gastric cancer. *World J Gastroenterol*, **11**, 791-6.