Hypermethylation of multiple Wnt antagonist genes in gastric neoplasia
Is H pylori infection blasting fuse?

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Abstract
Wnt antagonist genes hypermethylation has been found in several tumors. Accordingly, the events that occur during the progression of adenoma to carcinoma have been characterized and include activation of the Wnt-pathway. Further, gastric adenoma (GA) is a premalignant lesion of gastric adenocarcinoma (GAC). In this paper, we focused our interesting on Wnt signaling path function in the pathogenesis of GAC.

We compared the differences between low grade adenoma (LGA), high grade adenoma (HGA), GACs and corresponding normal gastric tissue (NGT). Specific indexes include the pathological characteristics of gastric neoplasia, Helicobacter pylori infection, β-catenin mutation status, and methylation status of Wnt antagonist genes.

There was significant difference of β-catenin expression in patient with NGT, LGA, HGA, and GAC, the results respectively were 4.2%, 41.7%, 83.3%, and 91.7%. Only 1 GACs was detected exon 3 of β-catenin mutation. Wnt antagonist genes mRNA expression levels, such as APC, sFRP-1, Wif-1, and Dikk-1, were significantly reduced in GAC. Promoter methylation levels of the 4 genes were significantly elevated in GAC and HGA compared to NGT and LGA. However, there was no significant difference between HGAs and GACs. The β-catenin abnormal expression was correlated with hypermethylation of these 4 genes. Multiple gene concurrent methylation phenomenon was increased from NGTs to GACs; the amount of methylation genes in GACs and HGAs was more than NGTs and LQAs. The more methylation of the above-mentioned genes, the more severity of local inflammation. The infection rate of H pylori was significantly higher in patient with HGA (66.7%, 16/24) and GAC (83.3%, 14/24) than in LGAs (16.7%, 4/24) (P = 0.024, P = 0.032). In addition, the present of H pylori also correlated with the β-catenin abnormal expression and the hypermethylation status of Wnt antagonist genes (P < .001). But other parameters in adenoma cases had no significantly related with infection of H pylori.

Hypermethylation of Wnt antagonist genes may have a tight relationship with gastric tumorigenesis. And these genes may increase the incidence of GAC. Additionally, H pylori may have promotion function in GA formation.

Abbreviations: DKK = DICKKOPF, GA = gastric adenoma, GAC = gastric adenocarcinoma, HGA = high-grade adenoma, IRS = immunoreactive score, LGA = low-grade adenoma, MSP = methylation-specific PCR, NGT = normal gastric tissue, H Pylori = Helicobacter pylori, sFRP-1 = secreted Frizzled-related proteins, TCF/LEF = T cell factor/lymphoid enhancer factor, USP = unmethylation-specific PCR, WIF-1 = Wnt inhibitory factor-1.

Keywords: gastric neoplasia, H pylori, hypermethylation, Wnt antagonist gene

1. Introduction
Currently, there was a common view that gastric cancer is one of the most common and deadly cancers in the worldwide. It is important to study early etiology and detection to develop effective methods for the diagnosis of fatal diseases. Gastric adenocarcinoma (GAC) usually progressed as the order of inflammation, metaplasia, dysplasia, and cancer.[1] There were 2 high risks of GACs: intestinal metaplasia (IM) and gastric epithelial dysplasia (GED), that was to say, neoplastic precursor lesions. A unique neoplastic growth characteristic of gastric adenoma (GA) is dysplastic epithelium localized polyloid proliferation. This growth characteristic tends to progress to infiltrating adenocarcinoma.[2] The current opinion considered that genetic epigenetic abnormalities may be involved in specific pre-malignant lesions. Its significance was not just confined to innocent atrophic gastritis.[3] Therefore, it helps to understand the progress from GA to GAC and identify pathogenic genes.

Literature has now proved that the key of adenomas to cancer was suppressor TP53 losses, as well as KRAS and Wnt pathway...
activation. The Wnt signaling pathway plays an important role in tumorigenesis. The classical Wnt signal pathway activation promoted the transcription of several target genes after the interaction of β-catenin accumulation and T-cell factor/lymph enhancer factor (TCF/LEF). Various other human malignancies had been proved they are conducted by the aberrant activation of Wnt signaling way.

Currently, we had found that the development and progression of GA were facilitated by methylation of the APC promoter rather than mutations of APC and β-catenin. Wnt antagonist genes such as APC, AXIN-2, Frizzled-related proteins (sFRPs), Wnt inhibitor (WIF-1) and DICKKOPFs (DKKs) in cancer may play an important role in the stabilization and accumulation of β-catenin. The methylation of the Wnt signaling pathway is moderately activated in physiological status. If there are aberrant methylation of above Wnt antagonist genes, Wnt/β-catenin signal way will be activated.

Recently, the wildly accepted view was that Helicobacter pylori (H pylori) infection have a close relation with gastric cancer (GC). H pylori infection contributes to epigenetic changes is known to increase the risk of GC in the future. In addition, some studies proved that E-cadherin gene, p16 gene, and APC gene methylation had closely correlation with H pylori infection. However, the bacterial role in GA development and progression is not clear.

Genes methylation was important in GA development because many genes have changed methylation patterns in the tumor. In this study, we evaluated

1. the changes about Wnt antagonist genes methylation status from GA to GAC,
2. the pathological characteristics correlation between gastric neoplasia, β-catenin mutational status, and Wnt antagonist genes expression and methylation status. And
3. the correlation between H pylori and the hypermethylation status of Wnt antagonist genes.

2. Methods

2.1. Specimens

Ethics committee of Jinling Hospital approved this study. There were 24 samples of primary GACs and corresponding normal gastric tissues (NGTs) in this study. And all the samples were obtained by radical or partial gastrectomy. The 48 cases which proceeding gastroscopic polypectomy were included. Among them, 24 cases were low-grade adenomas (LGAs) and other cases were high-grade adenomas (HGAs). Corresponding peridencomatous NGTs were obtained by endoscopic gastric biopsies. Histological analysis of selected biopsy materials showed that these samples contained 40% to 80% of epithelial tissues. Vienna Classification was the criterion for sample classification in this study. The updated Sydney System in peridencomatous NGTs was a standard for the assessment of inflammation, glandular atrophy, and intestinal metaplasia. First, the sample was immobilized by 10% buffered formalin (pH 7.0). Then the sample was embedded in paraffin wax. Sections (5 μm) of the sample was stained by hematoxylin and eosin for evaluation of histology. Except analysis, samples were stored at −80°C to ensure stability.

2.2. Immunohistochemistry

Immunohistochemical stain with anti-β-catenin (dilution 1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA) using a 5 μm thick section of formalin fixed and paraffin embedded (FFPE) sections and incubated 2 h at room temperature. Avidin-biotinperoxidase complex procedure (ABC standard; Vector Laboratories, Burlingame, CA, USA) was performed. Peroxidase activity was measured using 3,3′-diaminobenzidine tetrachloride as a substrate.

Cytoplasm and cell membrane were mostly possible expressed place of β-catenin. β-catenin immunoreactive score (IRS) was a wildly used semiquantitative compiled method. We used different scores to represent β-catenin staining intensity: if the score was 0, represented no staining; if the score was 1, represented weak staining; if the score was 2, represented moderate staining; and if the score was 3, represented strong staining. The positivity area evaluated by values. 0 represented focal or <10%, 1 represented 10% to 30%, 2 represented 30% to 50% and 3 represented >50%. The final IRS score was added score of staining intensity and score of percentage of positivity, the IRS score range from 0 to 6. The IRS pattern was divided into “weak” (IRS score <3) or “strong” (IRS score ≥3). Only when experiment result of immunohistochemical test showed strong membrane staining and weak cytoplasmic staining in the meantime, mean “normal” pattern. Except the only pattern, others all represented the “disordered” pattern. β-catenin immunostaining was evaluated by 2 independent observers which were blinded to patients’ clinical inspection results and local staging.

2.3. DNA and RNA isolation

In this study, TRIzol Reagent (Life Technologies, Breda, The Netherlands) was used to isolate DNA and RNA from cell lines. DNA from the FFPE material was isolated after the previously mentioned macro-dissection.

2.4. Analysis of the β-catenin gene

For β-catenin, genomic polymerase chain reaction (PCR) technology was used. Exon 3, a fragment previously thought to be activated by mutations, was amplified as method previously reported in the literature. High-fidelity Primaster DNA polymerase (HotStart version; TaKaRa, Dalian, China) was used in PCR procedure. Sequencing procedure was performed using an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, Calif., USA). To avoid mistakes, the above operation was repeated once more for the mutated sample.

2.5. Reverse transcription-PCR (RT-PCR) and quantitative RT-PCR (qRT-PCR) analysis

RT-PCR analysis of APC, sFRP-1, DKK-1, and WIF-1 expression was performed using cDNA synthesized from 1 μg of total RNA. 2% agarose gel was used to analyze the PCR products. qRT-PCR was performed using an iCycler with iQ SYBR Green Supermix (Bio-Rad) and the same gene-specific primers. The internal standard was β-actin. The relative amount of the test report was calculated by the formula $2^{-\Delta\Delta Ct}$ where ΔCt was the representative mean subtraction of β-actin Ct from the mean target Ct value.

2.6. Methylation-specific PCR (MSP)

Commercially available sodium bisulfite (Invitrogen, Carlsbad, CA, USA) was used to modify genomic DNA. APC, sFRP-1,
DKK-1, and WIF-1 were the targeted genes of this study. The first universal primer does not contain forward or reverse CpG site primers, and the amplified DNA fragment contains multiple promoter regions. The second round of nested MSP or unmethylated specific PCR (USP) was the use of a universal PCR product template. Primer sequences designed for MSP and USP Wnt antagonist genes have been reported.\textsuperscript{18} Preliminary tissue MSP semi-quantitative analysis of each primer PCR reaction cycle linear range. The PCR mixture was separated by 1.5% agarose gel electrophoresis. The gel contained ethidium bromide. For the conjugated structure of DNA bonds, PCR products were determined by UV light. For the specimen has a positive MSP bond, non-denaturing 12% polyacrylamide gel electrophoresis was used to separate MSP or USP and then determined relative methylation ratio. Image J software was used to calculate the corresponding each band area under the curve (AUC).\textsuperscript{4} Previously reported studies have determined the degree of correlated methylation [MSP ratio = MSP band density / (MSP band density + USP band density)].\textsuperscript{19,20} 2.7. Genetic analysis of H pylori.

The presence of the glmM gene was confirmed in DNA samples extracted from PT and GA by semi-nested PCR and sequencing as described above.\textsuperscript{21} Sequence analysis of the PCR products was performed using an ABI 310 Genetic Analyzer (Applied Biosystems). DNA base transformation was always made independently.

2.8. Statistical analysis

The result was described by statistical parameters mean and standard deviation (SD). And the final results were summarize by and bar charts. T test and Welch method (2-sided) was used to determine the distinguish of expression levels. Categorical variables were analyzed by χ2 or Fisher exact tests. Binomial logistic regression analysis was used to subordinately analyze categorical variables multivariate. \( P < 0.05 \) mean there existed statistically significant. All data were analyzed using SPSS software for Windows 13.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. Clinical and histological characteristics

Table 1 shows the patient’s clinical and histological features. Thirty men and 18 women participated in this study with an average age of 56 years (range: 23–82 years). The average size of adenomas is 9.4 mm, ranging from 6 to 25 mm, with adenomas more common than flattened glands. Most of the cases less than 10 mm. All peridenedematous NGTs divided into various grades of inflammation. Among the cases, 5 accompanied intestinal metaplasia while 3 accompanied glandular atrophy. The inflammation grade showed a strong correlation with the size of GAs (\( P = .000 \)) and grade (\( P = .006 \)).

3.2. Immunohistochemistry for β-catenin expression in different gastric epithelial tissues

To test Wnt/β-catenin signaling status in different gastric epithelial tissues, we firstly evaluated the localization of β-catenin in clinical tissues. In most NGT patients, β-catenin was mainly found in cell membranes and partly in cytoplasm. For LGAs, 14 of the 24 cases (58.3%), β-catenin mainly existed in the cell membrane, but the rest cases (41.7%) were acted as a disordered pattern. The β-catenin expression of these 10 cases was quite different from NGTs which expressed strongly in the cytoplasm. In the case of HGAs, β-catenin was found weakly in the cell membrane and strongly expressed in cytoplasm (20/24, 83.3%). Sometimes, weak nuclear staining could be observed. For the gastric cancer cases, β-catenin was found strong expression in cytoplasmic or nuclear rather than the cell membrane. Nearly all the inclusion cases could found this phenomenon (22/24, 91.7%) (Fig. 1). The difference of β-catenin expression between GACs and NGTs was statistically significant (\( P < .005 \)) (Table 2). However, the statistical differences did not exist between HGAs and GACs.

3.3. Mutation in exon 3 of β-catenin

None of the included cases of LGAs, HGAs, and NGTs was found mutations in exon 3 of β-catenin. Only 1 gene mutation was observed for 24 GACs (4.2%). By sequencing analysis, mutations in the in-frame 6bp deletions (delGGTGCC, Gly38Ala39) were found at codons 38 and 39.

3.4. mRNA expression of Wnt antagonist genes

The negative regulators that inhibit the Wnt/β-catenin signaling pathway, such as APC, DKK-1, sFRP-1, and WIF-1, were detected by qRT-PCR. Wnt antagonist gene expression decreased in HGAs and GACs (Fig. 2A). To confirm these results, qRT-PCR was also used to study mRNA expression levels. We also found that mRNA expression levels of GACs were significantly lower than LGAs (\( P = .000 \)) (Fig. 2B).

3.5. MSP for Wnt antagonist genes

In order to clarify whether the reduction of expression of the Wnt/β-catenin signaling pathway antagonist mentioned above was...
due to the methylation of CpG islands, we used MSP analysis to detect the methylation status of the CpG islands. The methylation levels of \( APC \), \( DKK-1 \), \( sFRP-1 \), and \( WIF-1 \) promoters in GAC and HGA were significantly increased compared to NGTs and LGAs (Table 3). For APC promoter methylation levels, there was no significant difference between NGTs and LGAs (\( P = 0.252 \)). For above genes’ promoter methylation levels, there were no significant differences between HGAs and GACs (\( P_{APC} = 1.0, P_{Dkk1} = 0.568, P_{sfrp1} = 0.561 \) and \( P_{wif1} = 0.251 \)). Promoter methylation of the 4 genes related with aberrant expression of \( \beta \)-catenin (Table 4). There were more concurrently genes methylated increased from NGTs to GACs. GACs and HGAs had more concurrently methylated genes in GACs and HGAs than NGTs and LGAs (\( P = 0.000 \)) (Fig. 3). About the number of simultaneously methylated genes, there was no significant difference between GACs and HGAs (\( P = 0.284 \)), or between NGTs and LGAs (\( P = 0.162 \)). However, there was a significant difference in the number of simultaneously methylated genes between LGAs and HGAs (\( P = 0.000 \)). The methylation level of these genes is correlated with the level of local inflammation (\( P = 0.000, r = 0.287 \)).

### 3.6. Presence of \( H \) pylori and correlation of the \( H \) pylori status and various factors

In this study, in addition to C\(^{13}\) respiration test, PCR were used to measure the \( H \) pylori \( glmM \) gene sequence as an indicator of the degree of \( H \) pylori infection. \( H \) pylori was detected in 37.5% (27/72) by C\(^{13}\) respiration test, 23.6% (17/72) by PCR, and 47.2%
The experiment result showed that the infection rate was significantly higher in HGAs (66.7%, 16/24) and GAC (58.5%, 14/24) than in LGAs (16.7%, 4/24) \((P_{\text{HGA-LGA}} = .024, P_{\text{GAC-LGA}} = .032)\), and there were similar features in β-catenin abnormal expression cases and Wnt antagonist gene hypermethylation cases. However, no other parameters of adenoma cases associated with \(H\ pylori\) infection were found in this study.

Table 3

| NGTs (%) | LGAs | HGAs | GCs | Total |
|----------|------|------|-----|-------|
| APC      | 1/24 (4.2) | 2/24 (8.3) | 3/24 (12.5) | 6/72 (8.3) | 3/24 (12.5) | 19/24 (79.2) | 20/24 (83.3) |
| DKK1     | 0     | 0    | 0   | 0     | 2/24 (8.3) | 9/24 (37.5) | 11/24 (45.8) |
| sFRP1    | 0     | 0    | 0   | 0     | 2/24 (8.3) | 14/24 (58.3) | 16/24 (66.7) |
| WIF      | 0     | 0    | 0   | 0     | 2/24 (8.3) | 12/24 (50.0) | 15/24 (62.5) |

NGTs:LGAs, \(P_{\text{APC}} = 0.252, P_{\text{DKK}} = P_{\text{sFRP}} = P_{\text{WIF}} = 0.013\).

HGAs:GACs, \(P_{\text{APC}} = 1.0, P_{\text{DKK}} = 0.568, P_{\text{sFRP}} = 0.561, P_{\text{WIF}} = 0.251\).

NGTs:HGAs and NGTs:GACs, \(P_{\text{APC}} = P_{\text{DKK}} = P_{\text{sFRP}} = P_{\text{WIF}} = 0.000\).

LGAs:GACs, \(P_{\text{APC}} = P_{\text{DKK}} = P_{\text{sFRP}} = P_{\text{WIF}} = 0.000, P_{\text{APC}} = 0.003\).

LGAs:HGAs, \(P_{\text{APC}} = P_{\text{DKK}} = P_{\text{sFRP}} = P_{\text{WIF}} = 0.000\).

GAC = primary gastric adenocarcinoma, HGA = high-grade adenoma, LGA = low-grade adenoma, NGT = corresponding normal gastric tissues.
4. Discussion

β-catenin played an important role in classical Wnt signaling pathway.[22] In the cytoplasm and nucleus, β-catenin/TCF/LEF transcription complex is formed by accumulated β-catenin and induces over-expression of the target gene.[23] In cases of NGTs, LGAs, HGAs and GACs, we detected ectopic expression rate of β-catenin with the result of 4.2%, 41.7%, 83.8%, and 91.7%, respectively. The difference between 3 gastric tumor tissues and NGT was significant (P < .001). However, there was no significant difference between HGAs and GAC (P = .682). The abnormal expressions of Wnt/β-catenin in GAs and GAC were similar because the histological characteristics of HGA were closer to GACs than LGAs.

Recent Wnt signaling pathways were research hotspots and several antagonists of this pathway had been identified. APC, sFRP-1, sFRP-2, sFRP-4, sFRP-5, Wif-1, and Dkk-3 acted as Wnt antagonist genes by binding Wnt molecules or low-density lipoprotein receptor related protein LRP5/LRP6 components receptor complex. Therefore, if Wnt antagonists losing its function, the Wnt pathway could be activated and induced ectopic expression of β-catenin. So far, Wnt antagonist genes downregulation not only find in GAC, but also in other malignancies, including bladder,[18,24,25] lung[26,27] and breast[28] cancer, chronic lymphocytic leukemia,[29] and even esophageal[30] carcinoma. In our study, mRNA expression levels of the examined Wnt-antagonist genes were significant lower in GACs than LGAs (P = .000). The correlation between Wnt antagonist gene methylation status and β-catenin ectopic expression was analyzed in this study for 4 gastric tissues. Compared to NGTs and LGAs, APC, DKK-1, sFRP-1, and WIF-1 promoter methylation levels were significantly elevated in GACs and HGAs (P < .001). In addition, for APC, DKK-1, sFRP-1, and WIF-1 promoter methylation levels, there were no significant differences between HGAs and GACs (PAPC = 1.0, PDKK1 = .568, PsFRP1 = .561, and PWIF1 = .251). Wnt antagonist genes promoter methylation and β-catenin ectopic expression existed remarkable consistency. This indicated that hypermethylation of the Wnt antagonist gene was one of the key mechanisms of transferring the β-catenin protein from the cell membrane to the nucleus. This may be mediated through the abnormal Wnt/β-catenin signal activation involved in the pathogenesis of GAs and GACs. The occurrence rate of genes concurrently methylated increased from

![The numbers of methylated genes in different gastric tissues](image-url)

**Figure 3.** Summary of the methylation profile in NGTs, LGAs, HGAs and GACs. Concurrently methylated genes increased from NGTs to GACs. GACs and HGAs had more concurrently methylated genes than NGTs and LGAs (P = .000). There was no significant difference in the number of concurrently methylated genes between GACs and HGAs (P = .284) or between NGTs and LGAs (P = .162). GAC = gastric adenocarcinoma; HGA = high-grade adenoma; LGA = low-grade adenoma; NGT = normal gastric tissue.
NGTs to GACs. Occurrence rate of concurrently methylated genes of GACs and HGAs were higher than NGTs and LGAs ($P = .000$). This finding may reflect that aberrant methylation of Wnt antagonist genes is a sign of precancerous lesions or early stage of gastric cancer and these genes involve in early initiation and transformation process.

In addition, the study reported by Guo et al.[13] indicated that most of the Wnt antagonist genes were methylated in some specific tumors. However, in some cases, methylation changes exist in tumor tissue as well as paired non-cancerous tissues. For the high sensitivity of MSP analysis, though para-tumor tissues could not find the character of cancer cell in the histomorphology, some genes hypermethylation presented to these samples or the premalignant lesions[130]. In the current study, the APC gene methylation in NGTs was found, it is quite different from other 3 Wnt antagonist genes. There did not exist significant difference between NGTs and LGAs ($P = .252$). Klumpp et al.[138] found that tumor suppressor gene p16 hypermethylation showed Barrett’s esophageal tumor progression, normal pathology later developed into dysplasia. Thus, APC genes epigenetic inactivation might be an early signal of tumorogenesis in GACs.

As far as we know, no studies on the promoter methylation and mRNA expression of Wnt antagonist genes in GA have been so far. But the relationship between Wnt antagonist genes promoter hypermethylation and gastric cancer/ esophageal carcinoma studied by some researcher.[30,33,34] Results of Yoshida and Saito[33] indicated that thirty percent of the GAs resected by endoscopy were associated with canceration, which suggested that the underlying pathogenesis of adenomas was similar to that of cancer. Another GAs study showed the risk of development to GCs was about 2.5% to 50%.[136,371] According to a recent opinion, GA is a more likely developed to cancer than atrophic gastritis alone.[13] Thus, we assess the relationship between the four Wnt antagonist genes of promoter hypermethylation, expression and mutation of β-catenin, and histologic features in GAs. The β-catenin aberrant expression exists in the NGTs, LGAs, and HGAs and the detected rate was 4.2%, 41.7%, and 83.3%, respectively ($P < .001$). Mutations of β-catenin exon 3 in LGA and GA were not detected. Compared with LGA, APC, DKK-1, sFRP-1, and WIF-1 promoter methylation levels were significantly increased in HGA ($P < .05$). There was a significant difference in the number of methylated genes between LGAs and HGAs ($P = .000$). Above mentioned genes methylation levels had a correlation with local inflammation level ($P = .000$, $r = .287$). The results indicated that gastric inflammation could be involved in the occurrence and development of GAs. This phenomenon also implies GA was a particularly premalignant lesion unlike glandular atrophy and intestinal epithelization.

CpG island promoter region hypermethylation is an epigenetic event. Several factors were found to cause this result, such as nitrosamines, tobacco tar, alcohol, high salt intake, and bacterial overgrowth. Especially $H$ pylori infection can cause hypermethylation of some tumor suppressor genes. The researchers came from Japan have shown that GAs are associated with $H$ pylori infection.[139] Study result of Komoto et al.[39] also showed that GAs often accompanied with $H$ pylori infection. Now there was much evidence showed that $H$ pylori infection was significantly associated with methylation of E-cadherin, p16 and APC genes. Additionally, eradication of $H$ pylori reversed the methylation of these genes in patients with chronic gastritis.[40] $H$ pylori of HGA patients (66.7%, 16/24) was significantly higher than that of LGAs (16.7%, 4/24) ($P = .024$) as compared with previous studies. Similar result was found between LGAs and GACs. We did not find statistically significant correlations with adenoma growth pattern, size and gene mutation, but there was significant correlation with hypermethylation and expression of every Wnt antagonist genes. Therefore, we believe that $H$ pylori plays a stimulatory role in GAs.

There are some drawbacks of this study, the result was based on small sample of inclusion patients and cancer-related genes. Thus, our results need larger multi-gene studies to prove. The exact function and interaction with other factors need more further work to elucidate. On the basement of further study, early diagnosis, prevention, and treatment strategies would be developed for GAC. And it was worth to further study the mechanism about $H$ pylori how to influence hypermethylation of multiple genes.

In conclusion, our data suggest that hypermethylation of various Wnt antagonist genes had a close relationship with irregular activation of Wnt signaling and finally caused gastric tumor. Thus, the methylation status of the Wnt antagonist gene plays an important mediator role in the progression of gastric cancer and may improve objective criteria, such as endoscopic mucosal resection. In this regard, hypermethylation testing can evolve as a diagnostic tool to determine the risk of histological progression of the GAC.

**Author contributions**

Author contributions: Wang ZK wrote the paper; Ye YQ designed and analyzed; Dan Liu collected the specimen; Xiaqiao Yang reviewed histopathology of specimen; Wang FY checked the article.

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**References**

[1] Correa P. A human model of gastric carcinogenesis. Cancer Res 1988;48:3554-60.

[2] Tsujitani S, Furusawa M, Hayashi I. Morphological factors aid in therapeutic decisions concerning gastric adenomas. Hepatogastroenterology 1992;39:56-8.

[3] Abrahamic SC, Montgomery EA, Singh VK, et al. Gastric adenomas: intestinal-type and gastric-type adenomas differ in the risk of adenocarcinoma and presence of background mucosal pathology. Am J Surg Pathol 2002;26:1276–85.

[4] Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–67.

[5] Anastas JN, Moon RT. WNT signaling pathways as therapeutic targets in cancer. Nat Rev Cancer 2013;13:11–26.

[6] Behrens J, vonKries JP, Kuhl M, et al. Functional interaction of β-catenin with the transcription factor LEF-1. Nature 1996;382:638–42.

[7] Leushacke M, Barker N, Lgr 5 and Lgr 6 as markers to study adult stem cell roles in self-renewal and cancer. Oncogene 2012;31:3009–22.

[8] Ramachandran J, Thavathiru E, Ramalingam S, et al. Wnt inhibitory factor 1 induces apoptosis and inhibits cervical cancer growth, invasion and angiogenesis in vivo. Oncogene 2012;31:2725–37.

[9] Schepeler T, Holm A, Halvey P, et al. Attenuation of the beta-catenin/TCF4 complex in colorectal cancer cells induces several growth-suppressive microRNAs that target cancer promoting genes. Oncogene 2012;31:2750–60.
[10] Zhen-Kai Wang, Jiong Liu, Chan Liu, et al. Hypermethylation of adenomatous polyposis coli gene promoter is associated with novel Wnt signaling pathway in gastric adenomas. J Gastroenterol Hepatol 2012; 27:1629–34.

[11] Makiita T, Nakazawa K, Mihara M, et al. High levels of aberrant DNA methylation in Helicobacter pylori-infected gastric mucosa and its possible association with gastric cancer risk. Clin Cancer Res 2006;12:989–95.

[12] Schlemper RJ, Riddell RH, Kato Y, et al. The Vienna classification of Gastrointestinal epithelial neoplasia. Gut 2000;47:251–5.

[13] Dixon MF, Genta RM, Yardley JH, et al. Classification and grading of gastritis. Am J Surg Pathol 1996;20:1161–81.

[14] Voorham QJ, Carvalho B, Spiertz AJ, et al. Chromosome 5q loss in colorectal flat adenomas. Clin Cancer Res 2012;18:4560–9.

[15] Enokida H, Shiina H, Igawa M, et al. CpG hypermethylation of MDR1 gene contributes to the pathogenesis and progression of human prostate cancer. Cancer Res 2004;64:5956–62.

[16] Stolte M. Clinical consequences of the endoscopic diagnosis of gastric neoplasia: correlations with histological gastritis and tumor histology. Am J Gastroenterol 1996;91:839–43.

[17] Stolte M. Clinical consequences of the endoscopic diagnosis of gastric polyps. Endoscopy 1995;27:32–7.

[18] Gotoda T, Saito D, Kondo H, et al. Endoscopic and histological reversibility of gastric adenoma after eradication of Helicobacter pylori. J Gastroenterol 1999;34(Suppl. 1):S1–6.

[19] Komoto K, Haruma K, Kamada T, et al. Helicobacter pylori infection and gastric neoplasia: correlations with histological gastritis and tumor histology. Am J Gastroenterol 1998;93:1271–6.

[20] Peri F, Cotugno R, Piepoli A, et al. Aberrant DNA methylation in nonneoplastic gastric mucosa of H. pylori infected patients and effect of eradication. Am J Gastroenterol 2007;102:1361–71.