Polymorphisms of Death Pathway Genes FAS and FASL and Risk of Premalignant Gastric Lesions

PING-I HSU¹, PEI-JUNG LU², E-MING WANG¹, LUO-PING GER², GIN-HO LO², FENG-WOEI TSAY¹, TAI-AN CHEN³, HSIAO-BAI YANG³, HUI-CHUN CHEN⁴, WEI-SEN LIN⁵ and KWOK-HUNG LAI¹

¹Division of Gastroenterology and General Medicine, Department of Medicine, and ²Department of Education and Research, Kaohsiung Veterans General Hospital and National Yang-Ming University, Kaohsiung; ³Department of Pathology, Ton-Yen General Hospital, Hsin-Chu County; ⁴Department of Radiation Oncology, Chang Gung Memorial Hospital - Kaohsiung Medical Center, College of Medicine, Chang Gung University, Kaohsiung; ⁵Department of the Health Care and Hospital Administration, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan, R.O.C.

Abstract. Background: Tumorigenesis is a multistep process that begins with the abrogation of normal controls of apoptosis and cell proliferation, and the FAS receptor-ligand system is a key regulator of apoptosis. The aim of this study was to investigate whether functional polymorphisms of death pathway genes (FAS and FASL) are associated with the development of gastric atrophy and intestinal metaplasia. Patients and Methods: Genotypes in the promoter regions of the FAS (−1377G/A and −670A/G) and FASL (−844T/C) genes of 101 healthy individuals and 86 gastric cancer patients were determined by PCR-RFLP. Additionally, gastric histological changes were examined according to the updated Sydney System. Results: The carriage of FASL −844C allele significantly increased the risk of atrophy in the gastric corpus, with an adjusted odds ratio (OR) of 5.0 [95% confidence interval (CI), 1.5-6.8]. There were no gene–gene interactions among FASL −844T/C, FAS −1377G/A and FAS −670A/G polymorphisms in developing premalignant gastric lesions. In the 109 individuals with Helicobacter pylori infection, carrying the FAS −1377A allele was a protective factor for developing intestinal metaplasia in the antrum (OR, 0.3; 95% CI, 0.1-0.9), while carrying the FASL −844C allele was a risk factor for developing gastric atrophy in the corpus (OR, 9.4; 95% CI, 1.7-53.4). Conclusion: FAS and FASL genotypes of the hosts are important determinants in the pathogenesis of gastric atrophy and intestinal metaplasia in H. pylori-infected individuals.

Worldwide, gastric cancer is the second most frequent cancer, killing more than one million people each year (1). There are two major types of gastric adenocarcinomas: intestinal and diffuse with the first being; the most frequent (2). In 1988, Correa et al. proposed a human model of gastric carcinogenesis based on epidemiological, pathological and clinical findings (3). They postulated that gastric carcinomas develop through a complex sequence of events from normal mucosa to superficial gastritis, atrophic gastritis, intestinal metaplasia, dysplasia and finally to intestinal-type adenocarcinoma. In the multistep carcinogenesis, corpus-predominant atrophy with loss of acid-secreting parietal cells appears to be a critical step, which facilitates gastric colonization by enteric bacteria with nitrate reductase and promotes the formation of carcinogenic nitrosamines (4).

The development of gastric cancer is associated with a multifactorial etiology (5, 6). Dietary and genetic factors traditionally have been given the greatest emphasis, but the role of Helicobacter pylori (H. pylori) infection in gastric carcinogenesis is now being appreciated. In 1994, the World Health Organization and International Agency for Research on Cancer classified H. pylori as a definite carcinogen (7). Long-term observation of H. pylori infection has provided evidence of a progression from H. pylori gastritis to atrophic gastritis, intestinal metaplasia, dysplasia and gastric adenocarcinoma (8). Two large-scaled cohort studies from Japan (9) and Taiwan (10) also indicated that gastric adenocarcinoma developed in persons infected with
H. pylori and not in uninfected persons, and intestinal metaplasia are important risk factors predicting subsequent development of gastric cancer in H. pylori-infected subjects (10).

From the biological and molecular points of view, tumorigenesis is a multistep process that begins with the abrogation of normal controls of apoptosis and cell proliferation. H. pylori possesses a number of virulent factors which damage the gastric mucosa and trigger apoptosis of gastric epithelial cells (11, 12). The enhanced apoptosis of gastric epithelial cells plays an important role in the pathogenesis of atrophic gastritis and gastric cancer. Recent studies demonstrated that H. pylori might induce apoptosis by activating FAS receptor-ligand system (13, 14). Wang et al. reported that H. pylori infection could induce the expression of FAS ligand (FASL) on the surface of infiltrating T-cells and increase FAS receptor expression on gastric epithelial cells. The FAS and FASL interactions then lead to gastric epithelial cell death (13). On the other hand, Rudi et al. reported that H. pylori-associated apoptosis of gastric epithelial cells not only involved the interactions between FAS-expressing gastric epithelial cells and FASL-expressing lymphocytes but might also occur by fratricide or suicide mediated by FAS and FASL interactions among gastric epithelial cells (15).

Recent studies have revealed that the FAS promoter is polymorphic, including a G to A substitution at −1377 bp and an A to G substitution at −670 bp (16-18). The FAS −1377A allele and FAS −670G allele disrupt Sp1 and STAT transcription factor binding sites, respectively, and thus diminish promoter activity and decrease FAS gene expression (18). The promoter of the FASL gene also has a functional single-nucleotide polymorphism – a T or C at position −844, which is located in a binding motif of CAAT transcription factor (19). The basal expression of FASL in individuals carrying the FASL −844C allele is higher than that in those carrying the FASL −844T allele (19).

Because the abrogation of normal controls for apoptotic cell death plays an important role in the development of cancer, we designed this study to investigate whether functional polymorphisms of FAS and FASL death pathway genes are associated with the development of premalignant gastric lesions, gland atrophy and intestinal metaplasia.

**Patients and Methods**

**Study participants.** One hundred and one consecutive asymptomatic healthy individuals and 86 patients with gastric cancer were included in this study. The healthy individuals were enrolled from our health examination clinics, for which panendoscopy was a routine examination of the general health checkup because gastric cancer incidence is high in our country. The gastric cancer patients underwent endoscopy for upper gastrointestinal symptoms. The diagnosis of gastric cancer was confirmed by gastric biopsy. To minimize ethnic bias, participants and gastric cancer patients were Han Chinese; aboriginal and alien populations were excluded. Exclusion criteria for both groups included: (i) history of esophageal, gastric or duodenal ulcer, (ii) previous history of anti-H. pylori therapy, (iii) use of non-steroidal anti-inflammatory drug or proton pump inhibitors within one month of endoscopy, and (iv) serious medical illness.

**Study design.** Endoscopies were performed with an Olympus GIF XV10 and GIF QX200 (Olympus Corp., Tokyo, Japan). During endoscopy, biopsies over the antrum and corpus were performed for rapid urease testing and histological examination. Prior to endoscopy, venous blood was drawn for serological testing, as well as FAS and FASL genotyping. The diagnosis of H. pylori infection was based on at least one positive result of a rapid urease test and serological assay.

The following data were recorded for each participant: age, gender, cigarette smoking, alcohol consumption and dietary history for consumption of salty food, fermented food, fresh vegetables and fruits. The study was approved by the Medical Research Committee of the Kaohsiung Veterans General Hospital. All patients and controls gave informed consent.

**Histology.** A histological examination of the stomach was carried out during endoscopy for those who provided informed consent for topographical histopathological study. Two specimens were taken from each of the antrum (pyloric gland area) and corpus (fundic gland area) at standard topographic sites. The biopsy specimens were fixed in 10% buffered formalin, embedded in paraffin and sectioned. The sections were stained with a haematoxylin and eosin stain and a modified Giemsa stain as previously described (20). Sections were examined blinded to the patient’s clinical diagnosis. The scores of H. pylori density, acute inflammation (neutrophil infiltration), chronic inflammation (mononuclear cell infiltration), glandular atrophy and intestinal metaplasia were graded from 0 to 3 as described by the Updated Sydney system (21).

**Rapid urease test.** The rapid urease test was performed according to our previous studies (22). Each biopsy specimen was placed immediately in 1 ml of a 10% solution of urea in deionized water (pH 6.8) to which two drops of 1% phenol red solution had been added and incubated at 37°C for up to 24 hours. If the yellowish color around the area of inserted specimen changed to bright pink within the 24-hour limit, the urease test was considered positive. In our laboratory, the sensitivity and specificity of the rapid urease test were 96% and 91%, respectively (23).

**Serology.** Serology used an indirect solid-phase immunochromatographic kit (ASSURETM H. pylori rapid test; Genelabs Diagnostics, Cavendish Singapore Science Park, Singapore). The sensitivity and specificity of the assay were 96% and 92%, respectively, according to the manufacturer’s instructions.

**FAS and FASL genotyping.** Genomic DNA was extracted from 3 ml of whole blood by the use of a QIAamp DNA Extraction Mini Kit (QIAGEN Inc., Valencia, CA, USA). The FAS and FASL polymorphism analysis was performed using a polymerase chain reaction-based restriction fragment length polymorphism method (PCR-RFLP). The primers used were FAS −1377G/A; 5'-TGTG TGCAACAGGCTGGCCGC-3' (forward) and 5'-TGCATCTGTC
ACTGCACTTACCACCA-3’ (reverse); FAS –670A/G polymorphism: 5’-ATAGCTGGGGCTATGCGATT-3’ (forward) and 5’-CATTTGCTCCAT-3’ (reverse); FASL promoter region containing the –844T/C polymorphism: 5’-CAGCTACTCGGGACTGGGCTGTCCAT-3’ (reverse); 5’-ATAGCTGGGGCTATGCGATT-3’ (forward) and 5’-CATTTGCTCCAT-3’ (reverse); FAS –1377G/A, FASL –844T/C polymorphisms, the –844T/C polymorphism, 1 minute at 74°C for 1 minute, and a final extension step of 10 minutes at 74°C.

The gastric cancer patients were significantly older than those in healthy controls (p=0.001). Additionally, the rates of male gender, high intake of salty food, high intake of fermented food, H. pylori infection, atrophy and intestinal metaplasia of the antrum, atrophy and intestinal metaplasia of the corpus in gastric cancer patients were significantly higher than those in healthy controls (p<0.001, 0.013, <0.001, 0.019, <0.001, <0.001, <0.001 and <0.001, respectively). Gastric cancer patients had a lower intake of vegetables and fruits, fresh vegetables and fruits, and H. pylori status.

**Results**

**Characteristics of the patients.** Table I shows the demographic characteristics of the enrolled participants. The gastric cancer patients were significantly older than the healthy controls (p<0.001). Additionally, the rates of male gender, high intake of salty food, high intake of fermented food, H. pylori infection, atrophy and intestinal metaplasia of the antrum, atrophy and intestinal metaplasia of the corpus in gastric cancer patients were significantly higher than those in healthy controls (p=0.001, 0.013, <0.001, 0.019, <0.001, <0.001, <0.001 and <0.001, respectively). Gastric cancer patients had a lower intake of vegetables than healthy controls (p=0.012). With regard to the polymorphisms of death pathway genes, gastric cancer patients had a lower intake of vegetables than healthy controls (p=0.012). With regard to the polymorphisms of death pathway genes, gastric cancer patients had a lower intake of vegetables than healthy controls (p=0.012).
patients had a higher frequency of FASL –844 CC genotype than healthy subjects (55% vs. 43%), but the difference did not reach statistical significance (p=0.119). The genotype frequencies of the FAS gene at position –1377 and FAS –670 were similar between groups.

Impact of the genetic polymorphisms of FAS and FASL genes on histological gastritis. Table II displays the relationships between genetic polymorphism of FAS –1377 and histological gastritis. No differences in the scores of neutrophil infiltration, mononuclear cell infiltration, gland atrophy or intestinal metaplasia of gastric mucosa were found among the subjects with GG, GA and AA genotypes. Similarly, there were no associations between the genetic polymorphism of FAS –670 and any parameters of histological gastritis (Table III). With regard to genetic polymorphism of FASL –844, individuals with CT and CC genotypes displayed higher scores of gland atrophy in the corpus than did individuals with the TT genotype (Table IV; 0.82±0.11 and 0.82±0.11 vs. 0.24±0.12, both p=0.01). Additionally, individuals with CT and CC genotypes also had higher scores of intestinal metaplasia in the corpus than did individuals with the TT genotype (0.43±0.11 and 0.59±0.11 vs. 0.29±0.14), but the differences were not statistically significant.

Gene–gene interactions of FAS and FASL polymorphisms in the development of premalignant gastric lesions. Table V shows the gene–gene interaction of FASL –844T/C and FAS –1377G/A polymorphisms in the development of gland atrophy of the corpus. The FASL –844C allele was associated with an increased risk of gland atrophy in the corpus [95% confidence intervals (CI): 1.1-33.5]. The FAS –1377A allele did not significantly increase the risk of gland atrophy (95% CI: 0.2-15.1). FASL –844C allele carriers who possessed the FAS –1377A allele did not have a higher risk...
of gland atrophy than did FASL –844C allele carriers without the FAS –1377A allele [odds ratio (OR): 5.1 vs. 6.1]. Therefore, no synergistic interactions between the FASL –844C and FAS –1377A alleles for developing gastric atrophy of the corpus existed. Further analysis revealed no gene-gene interactions of FASL and FAS polymorphisms for the development of gland atrophy or intestinal metaplasia in the antrum and corpus (data not shown).

**Independent risk factors for the development of premalignant gastric lesions.** Unconditional logistic regression analysis was used to assess the relationships between the genetic polymorphisms of death pathway genes and the risk of premalignant gastric lesions. Neither the FAS –1377A allele nor the FAS –670G allele was associated with an increased risk for either gland atrophy or intestinal metaplasia in the antrum or corpus. In contrast, multivariate analysis revealed that the FASL –844C allele was an independent risk factor for developing gland atrophy in the corpus, with an adjusted OR of 5.0 (95% CI: 1.5-16.8). The other independent risk factors for gland atrophy in the corpus were advanced age and H. pylori infection (Table VI; 95% CI: 1.7-7.6 and 1.3-5.4, respectively).

**Impacts of the genetic polymorphisms of FAS and FASL genes on H. pylori-related gastritis.** To investigate the impacts of death pathway genes on H. pylori-related gastritis, we further examined the relationships between the genetic polymorphisms of FAS and FASL genes and premalignant gastric lesions in 109 H. pylori-infected individuals (Table VII). Multivariate analysis disclosed that carrying the FASL –1377A allele was a protective factor for the development of intestinal metaplasia in the antrum (OR: 0.3; 95% CI: 0.1-0.9), while carrying the FASL –844C allele was a risk factor for gland atrophy in the corpus (OR: 9.4; 95% CI: 1.7-53.4). Figure 1 illustrates how the host FASL –844 genotypes impact H. pylori-related gastritis. Amongst the H. pylori-infected individuals, the atrophic scores of the corpus in the individuals with FASL –844TT and the FASL –844C allele carriers were 0.25±0.18 and 2.44±0.08, respectively. The FASL –844C allele carriers exhibited higher scores of gland atrophy in the corpus than non-carriers (p=0.024).

**Discussion**

The FAS receptor-ligand system is a key regulator of apoptotic cell death. This molecular pathological study investigated whether genetic polymorphisms in the death
pathway genes FAS and FASL were associated with the development of premalignant gastric lesions. The analysis demonstrated that the carriage of the FASL –844C allele significantly increased the risk of atrophy in the gastric corpus with an adjusted OR of 5.0 (95% CI, 1.5-6.8). Both individuals with FASL –844 CT and CC genotypes displayed higher scores of gland atrophy in the corpus than did individuals with the TT genotype. We next examined whether there was a statistical interaction between the FAS and FASL genotypes for developing gastric atrophy or intestinal metaplasia. Our data indicated that there were no synergistic effects for developing any premalignant gastric lesions between the death pathway genes FAS and FASL. To the best of our knowledge, this study is the first to verify that functional polymorphism of the FASL gene is an important determinant in the development of premalignant gastric lesions.

Our results demonstrating an association between FASL –844C allele and the risk of developing gastric atrophy of the corpus are biologically plausible. Firstly, the 5’ flanking of the FASL gene promoter is comprised of many cis-regulatory response elements acting as binding sites for various transcription factors. The promoter of FASL at position -844 is located in a binding motif for a transcription factor – CAAT (23). Basal expression of FASL in individuals with the FASL –844C allele is significantly higher than that in individuals with FASL -844T allele (23). Given the role of FASL in apoptosis, one might expect that the enhanced expression of FASL on the surface of infiltrating T-cells in individuals carrying FASL –844C alleles would lead to an increase of apoptosis of gastric epithelial cells and progression of gland atrophy following the exposure of the gastric mucosa to detrimental factors, such as H. pylori infection and a high salt diet. Secondly, an association has been reported between the FASL –844C allele and risks of various tumors including ovarian (25), cervical (26), bladder (27) and esophageal cancer (24). Additionally, the FASL –844CC genotype has been linked to autoimmune diseases, such as systemic lupus erythematosus, characterized by accelerated FAS/FASL-mediated apoptosis of lymphocytes (19).

Tumorigenesis of gastric cancer is attributable to the interactions between environmental and genetic factors. H. pylori infection is one of the most important environmental causes for gastric carcinogenesis. Colonization of H. pylori in the stomach would induce local and systemic immune responses with increased gastric T-cell infiltration (28). The infiltrating T-cells play an important role in inducing gastric apoptosis by a FAS/FASL interaction (13, 14, 29). From the biological and molecular points of view, persistent H. pylori infection would cause repetitive apoptosis of gastric epithelial cells and therefore enhance subsequent cellular degeneration and finally lead to somatic mutations of critical genes during repair processes (30). In this study, we further investigated the impacts of functional polymorphisms of FAS and FASL genes on H. pylori-related gastritis. Our data disclosed that carrying the FASL –844C allele was a risk factor for developing gland atrophy of the corpus in H. pylori-infected subjects with an OR of 9.4. In contrast, carrying the FAS –1377A allele was a protective factor for the development of intestinal metaplasia in the antrum with an OR of 0.3. Currently, we have no definite rationale to explain the inverse associations between the FAS –1377A allele and risk of premalignant gastric lesions, but the FAS –1377 G/A polymorphism occurs in the promoter region within the Sp1 transcription factor binding site (18). Reduced expression of FAS in gastric epithelial cells carrying the FAS –1377A allele is expected, and H. pylori-infected individuals with FAS –1377A allele might have lower apoptosis and cellular regeneration of gastric epithelial cells compared with the subjects without the FAS –1377A allele.

Gastric atrophy and intestinal metaplasia are well-known precancerous lesions and it is important to identify individuals prone to develop these premalignant gastric lesions following H. pylori infection. In this study, we investigated whether functional polymorphisms of death pathway genes (FAS and FASL) are associated with the development of gland atrophy and intestinal metaplasia. However, several limitations of this study exist. Firstly, sampling error was unavoidable since precancerous lesions might exist in focal areas of the stomach and endoscopic biopsies were only taken at several standard sites. Secondly, bacterial virulent factors were reported to be associated with the development of gland atrophy and intestinal metaplasia (31), but they were not investigated in the current study. Therefore, it would be worthwhile to conduct studies to examine the combined effects of functional polymorphisms of death pathway genes and bacterial virulent factors in the pathogenesis of premalignant gastric lesions.

In conclusion, our work verifies that FAS and FASL genotypes are critical determinants in the development of atrophic gastritis and intestinal metaplasia in H. pylori-infected individuals.

Acknowledgements

This study was supported by research grants VGHKS95-115-1 from the Research Foundation of Kaohsiung Veterans General Hospital and NSC-95-2314-B-075B-002 from the National Science Council, Taiwan, R.O.C. The authors express their deep appreciation to Dr. Chung-Jen Wu and Miss Chieu-Shia Hsieh for their generous support.

References

Hsu et al: FAS and FASL Polymorphisms in Gastric Atrophy