Research Paper

Aberrant methylation of *FAT4* and *SOX11* in peripheral blood leukocytes and their association with gastric cancer risk

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Abstract

Background: Aberrant DNA methylation, especially tumor suppressor gene hypermethylation, is a well-recognized biomarker of initial tumorogenesis stages. *FAT4* and *SOX11* are putative tumor suppressor genes and can be down-regulated by hypermethylation in various cancers tissues. However, in peripheral blood leukocytes, the association between these two genes methylation status, as well as the effects of gene-environment interactions, and gastric cancer (GC) risk remain unclear.

Methods: A hospital-based case-control study including 375 cases and 394 controls was conducted. Peripheral blood leukocytes DNA methylation status were detected by methylation-sensitive high-resolution melting (MS-HRM) assay. Logistic regression was adopted to analyze the relationship of *FAT4* and *SOX11* methylation with GC susceptibility.

Results: Positive methylation (Pm) and total positive methylation (Tpm) of *FAT4* were significantly increased the risk of GC (OR = 2.204, 95% CI: 1.168-4.159, *P* = 0.015; OR = 1.583, 95% CI: 1.031-2.430, *P* = 0.036, respectively). Compared with controls, cases exhibited higher *SOX11* Pm frequencies with OR of 2.530 (95% CI: 1.289-4.969, *P* = 0.007). Nonetheless, no statistically significant association between *SOX11* Tpm and GC risk was observed. Additionally, interactions between *FAT4* Tpm and increased consumption of freshwater fish (≥1 times/week) displayed an antagonistic effect on GC (OR = 0.328, 95% CI: 0.142-0.762, *P* = 0.009), and high salt intake interacted with *SOX11* Tpm also showed statistically significant (OR = 0.490, 95% CI: 0.242-0.995, *P* = 0.048).

Conclusions: *FAT4* aberrant methylation in peripheral blood leukocytes and gene-environment interactions were associated with the risk of GC, while *SOX11* was controversial and needed to be more investigated.

Key words: DNA methylation, peripheral blood leukocytes, gastric cancer, gene-environment interaction.

Introduction

Gastric cancer (GC), a neoplasm with aggressive and highly proliferative condition, contributes considerable healthy burden from a global perspective. According to the Globocan 2012, GC is

the fifth most common malignancy and ranks as the third leading cause of cancer-related death in the world [1]. In China, GC has become the second leading cause of cancer death. According to the statistics, 679,100 new GC cases and 498,000 cancer deaths are estimated to occur in 2015 [2]. Although GC showed a decreasing incidence and mortality trend in recent years, population growth and aging will still lead to a large number of new cases in China [2].

It is well recognized that the development of GC is a multifactorial induction process, genetic and epigenetic alterations are involved in it as critical contributors. Nowadays, much attention has been paid to epigenetic alterations, which can induce heritable changes in gene expression without any alterations in the primary DNA sequence [3]. DNA methylation, which plays a key role in gene transcription and expression programming, is one of the most extensively studied epigenetic alterations. Aberrant DNA methylation frequently occurs at CpG islands within promoter regions, leading to transcription inhibition and gene inactivation, and is recognized as a critical component of the mechanism underlying tumorigenesis [4]. Several studies have demonstrated that aberrant DNA methylation can be a driver event in the pathogenesis of cancers GC [5-7].

FAT4, which located at the apical point of the Hippo signaling pathway, controls the size of organs by modulating cell growth, proliferation, and apoptosis [8, 9]. *SOX11* encodes a member of the group C SOX (SRY-related HMG-box) transcription factor family that participates in cell proliferation, embryonic neurogenesis and tissue remodeling [10]. Both *SOX11* and *FAT4* are supposed to be potential tumor suppressor genes and can be down-regulated by promoter aberrant methylation modification in several cancers, including GC [11-14]. It has been also reported that *FAT4* functions as a GC suppressor gene by modulating Wnt/*β*-catenin signaling [15], and aberrant *SOX11* promoter methylation is related to the clinical outcome of GC [16, 17].

Multiple environmental factors, including *H. pylori* infection, can profoundly influence tumorigenesis [18]. Recently, growing evidences have revealed that environmental factors may induce epigenetic alterations to further increase cancer susceptibility [19-23]. In addition, previous studies frequently focused on tumor tissues to examine DNA methylation status. However, in comparison with tissues which usually display incomplete hetero-geneity information, blood samples may capture the entire heterogeneity of cancer [24]. DNA methylation in the peripheral blood leukocytes is also linked to the susceptibility to several cancers according to recent studies [25-27]. Therefore, we carried out this case-control study to detect the methylation status of *FAT4* and *SOX11* in peripheral blood leukocytes and investigate the association between genes methyl-ation, gene-environment interactions, and GC risk.

Materials and methods

Study subjects

A hospital-based case-control study with 375 GC cases and 394 cancer-free controls was carried out. All newly pathologically confirmed GC cases without other digestive system tumors were recruited from the Third Affiliated Clinical Hospital of Harbin Medical University from 2010 and 2012. The controls comprised 334 ophthalmic and orthopedic patients of the Second Affiliated Hospital of Harbin Medical University, 33 neurological patients of the Fourth Affiliated Hospital of Harbin Medical University and 27 healthy individuals who underwent physical examinations at the Center for Disease Control of Xiangfang District, Harbin between 2010 and 2013. All control subjects with a history of malignant tumors or gastrointestinal diseases were excluded. Approxi-mately 5 ml blood samples were obtained and every participant completed a face-to-face questionnaire after obtaining informed written consent according to the protocols approved by the Human Research and Ethics Committee of Harbin Medical University. The overall response rate for cases and controls were approximately 90%.

DNA extraction and bisulfite modification

Genomic DNA was extracted and then bisulfite-modified using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) and EpiTect Plus DNA Bisulfite Kit (Qiagen) according to the manufacturer's instructions, respectively. DNA quantity was measured by the Nanodrop 2000 Spectrophotometer (Thermo scientific) and bisulfite-modified DNA was stored at -20℃ until use.

Serologic tests of the H.pylory by ELISA

The serologic test for *H.pylori* IgG antibodies were performed in duplicate using an enzyme immunoassay kit (IBL, German). The sensitivity and specificity of the kit were all greater than 95% according to the manufacturer's instructions.

Methylation-sensitive high-resolution melting (MS-HRM) assay

Primers used for amplifying *FAT4* gene CpG rich regions were designed using Primer Premier 5.0 software. For *SOX11*, primers were selected from previously published study [28]. The primer sequences used are listed in Table S1. PCR

amplification and MS-HRM assay was performed on a LightCycler480 (Roche Applied Science, Mannheim, Germany) equipped with Gene Scanning software (version 2.0) to detect and analyze the methylation status of genes. The final volume of each reaction system was 5μl, including 2.5μl of LightCycler480 High Resolution Melting Master Mix (Roche), 0.5μl of sodium bisulfite-modified template DNA, 0.1 μl of each forward and reverse primer, and 0.6 μl $MgCl_2$. A series of methylated DNA standards (100%, 2%, 1%, 0.5% and 0% methylated DNA) were constructed by mixing universal unmethylated (0% methylated) and methylated (100% methylated) human whole genomic DNA samples (Zymo Research). The profiles of normalized melting curves and melting peaks of the MS-HRM assay for each gene were shown in Figure 1-2. The methylation status of genes was determined by comparing the curve of each sample with the standards curves. Then, 0% and 0.5% methylated DNA served as the cut-off values to distinguish negative methylation (Nm) and positive methylation (Pm), respectively. Heterogeneous methylation (Hm)

was defined when samples melting curves presented a wider peak than the unmethylation peak or a methylation peak but before the real methylation melting profile. Total positive methylation (Tpm) was defined as Hm and Pm. In addition, DNA-free distilled water was used as blank control (no-template control) in each batch, and second trials were conducted for the unsure results.

Statistical analysis

The chi-square test was applied to estimate categorical variables. Univariate and multivariate logistic regression analyses were used to assess odd ratios (OR) and 95% confidence interval (CI) for the associations between gene-specific DNA methylation, environmental factors and GC risk. Multivariable logistic regression model with backward conditional selection method was used to select GC-associated environmental risk factors (*P* values of 0.05 and 0.10 were specified as the thresholds for entry and removal of variables, respectively). The combined effects of environmental factors and gene-specific DNA
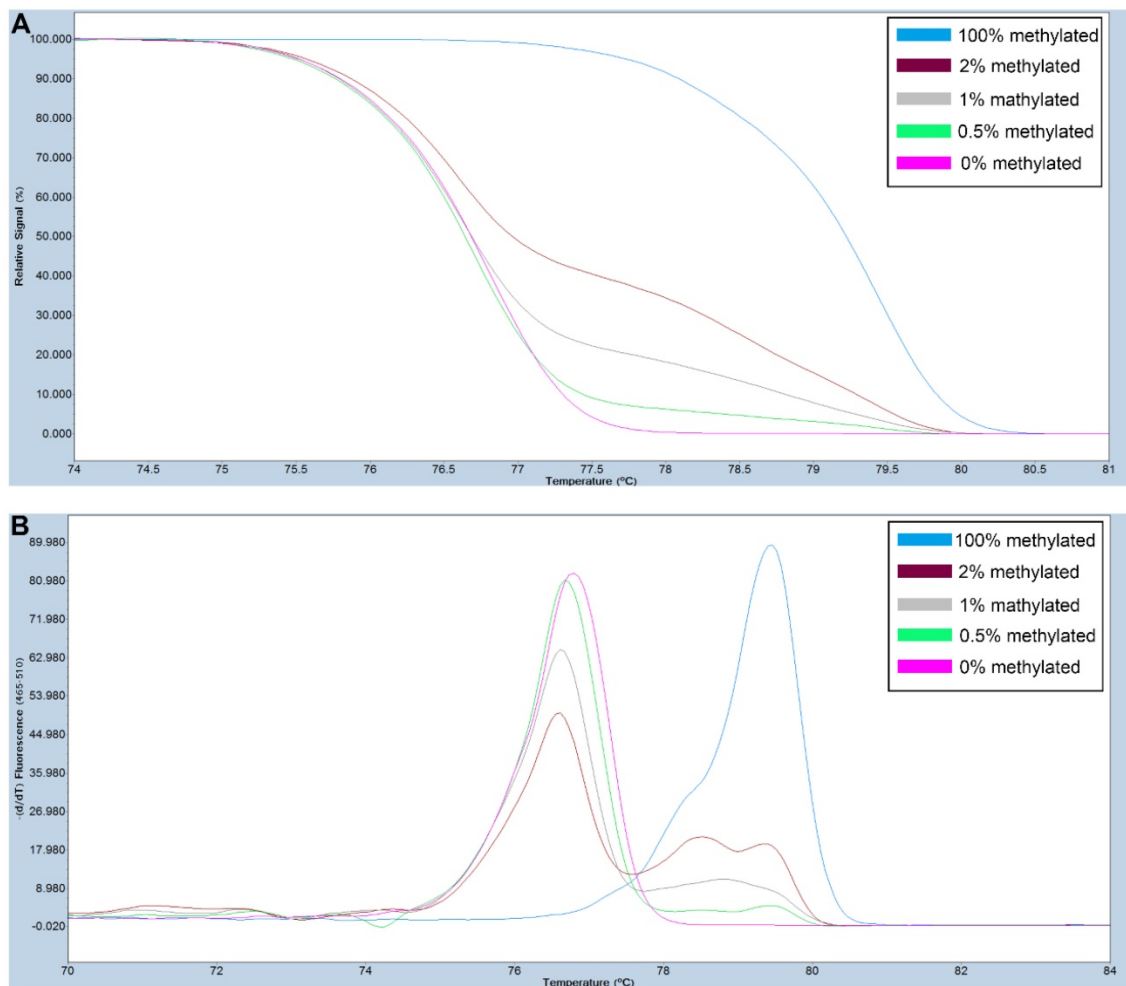

Figure 1. A series of methylated DNA standards (100%, 2%, 1%, 0.5% and 0% methylated DNA) was used for *FAT4*. A: Normalized melting curves of the MS-HRM assay for *FAT4*. B: Melting peaks were generated by taking the negative derivative (d) of the melting curve data divided by the derivative with respect to time −(d/dT).
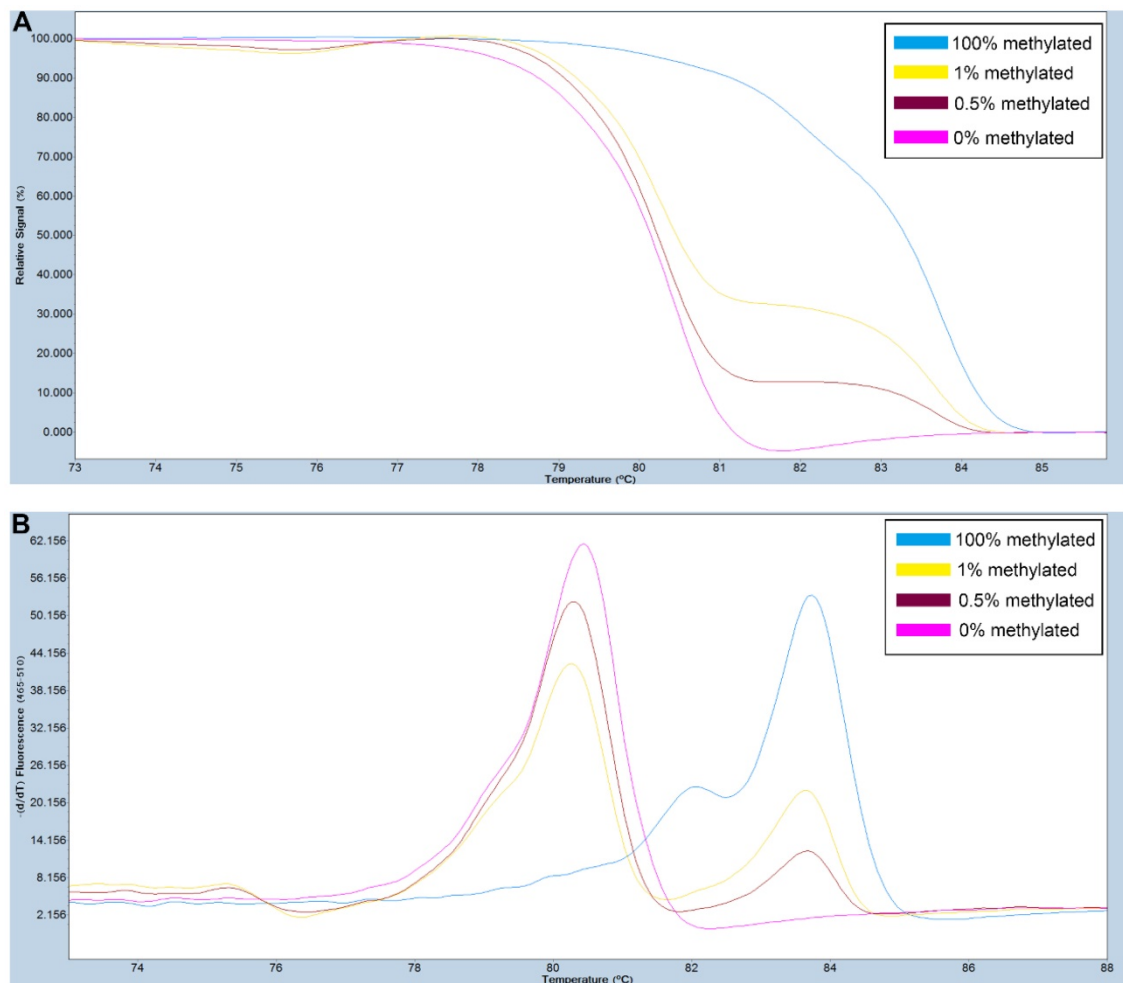
Figure 2. A series of methylated DNA standards (100%, 1%, 0.5% and 0% methylated DNA) was used for *SOX11*. A: Normalized melting curves of the MS-HRM assay for *SOX11*. B: Melting peaks were generated by taking the negative derivative (d) of the melting curve data divided by the derivative with respect to time −(d/dT).

methylation were calculated by crossover analysis. Gene-environment interactions on the risk of GC were evaluated on a multiplicative scale with a product-term coefficient using multivariable logistic regression. The relationships of gene methylation and environmental factors were further explored by multivariable logistic regression. All statistical analyses were performed using SPSS software version 19.0, with *P*-values of < 0.05 considered statistically significant.

Results

Characteristics of subjects

A total of 375 cases and 394 controls were enrolled in this study. The basic characteristics of these subjects are presented in Table 1. The distribution of BMI and occupation between cases and controls was statistically different (*P* < 0.001). The proportion of members with family history of GC in cases (12.8%) was higher than that in controls (1.8%; *P* < 0.001).

Table 1. Characteristics of subjects.

Characteristics		Case (%)	Control (%)	P
Gender	Male	285 (76.0)	298 (75.6)	0.906
	Female	90 (24.0)	96 (24.4)	
Age (years)	<60	200 (53.5)	202 (51.3)	0.540
	≥60	174 (46.5)	192 (48.7)	
Educational level	Primary school or below	110 (29.3)	114 (28.9)	0.996
	Middle school	139 (37.1)	147 (37.3)	
	Senior school	78 (20.8)	84 (21.3)	
	College or above	48 (12.8)	49 (12.4)	
BMI (kg/m²)	≤18.5	42 (11.3)	24 (6.2)	<0.001*
	18.5-23.00	181 (48.5)	133 (34.5)	
	≥23.00	150 (40.2)	229 (59.3)	
Nation	Han	361 (96.3)	372 (94.9)	0.357
	Other	14 (3.7)	20 (5.1)	
Regions	Urban	226 (60.6)	238 (60.6)	0.993
	Rural	147 (39.4)	155 (39.4)	
Occupation	Non-manual worker	135 (36.2)	31 (8.1)	<0.001*
	Light manual worker	76 (20.4)	157 (41.1)	
	Medium and heavy manual worker	162 (43.4)	194 (50.8)	
Family history of GC	No	326 (87.2)	379 (98.2)	<0.001*
	Yes	48 (12.8)	7 (1.8)	

BMI, body mass index; GC, gastric cancer. *Statistically significant.

Associations between environmental factors and GC risk

Multivariate logistic regression analyses displayed that several environmental factors, including irregular diet, high salt intake, overnight food, freshwater fish, fried food, smoking, drinking and *H.pylori* infection were significantly associated with increased GC risk ($P < 0.01$). In contrast, increased consumption of green vegetables, bean products and garlic could decrease GC risk with ORs of 0.270, 0.610, and 0.306 (Table S2). Backward conditional selection results suggested that irregular diet, salt, green vegetables, freshwater fish, garlic, *H.pylori*, drinking, overnight food and fried food were associated with GC risk ($P < 0.05$; Table S3).

Association between the methylation status of *FAT4*, *SOX11* and GC risk

Compared with Nm, subjects with *FAT4* Pm and Tpm showed 2.204-fold and 1.583-fold increased risk of GC (95% CI: 1.168-4.159, $P = 0.015$ and 95% CI: 1.031-2.430, $P = 0.036$, respectively). Individuals with *FAT4* Hm could have a 1.695-fold increased risk of GC before adjustment (95% CI: 1.239-2.319, $P = 0.001$). Statistically significant association between *SOX11* Pm and GC risk was observed (OR = 2.530, 95% CI: 1.289-4.969, $P = 0.007$), while no significant differences of *SOX11* Hm and Tpm between cases and controls were found (Table 2).

Stratified analyses were further conducted in this research. The results of age-stratified analyses revealed that *SOX11* Pm and Tpm was associated with increased risk of GC only in the older group (≥ 60 years, OR = 3.342, 95% CI: 1.480-7.545, $P = 0.004$ and OR = 1.721, 95% CI: 1.008-2.936, $P = 0.047$, respectively; Table 3). Moreover, in both the positive and negative *H. pylori* infection groups, individuals with *SOX11* Pm conferred an increased risk of GC after adjusting for gender, BMI, occupation and family history of GC (OR = 2.284, 95% CI: 1.053-4.952, $P=0.036$ and OR = 3.323, 95% CI: 1.385-7.973, $P = 0.007$, respectively). Non-significant associations of *FAT4* and *SOX11* Hm with GC risk were observed in these two stratums (Table 4).

Relationships between *FAT4*, *SOX11* methylation and environmental factors

The relationships between *FAT4*, *SOX11* methylation and environmental factors were explored in all the 375 GC cases and 394 controls. As shown in Table S4, consumption of green vegetables (≥ 250g/day) and freshwater fish (≥ 1 times/week) decreased the risk of *FAT4* methylation (OR = 0.450, 95% CI: 0.262-0.772, $P = 0.004$ and OR = 0.661, 95% CI: 0.458-0.953, $P = 0.027$, respectively), while drinking increased the risk of *FAT4* and *SOX11* methylation (OR = 1.480, 95% CI: 1.025-2.136, $P = 0.036$ and OR = 1.835, 95% CI: 1.272-2.646, $P = 0.001$, respectively).

Table 2. Association between methylation status of *FAT4*, *SOX11* and GC risk.

Methylation status		Control (%)	Case (%)	OR (95% CI)	P	OR[a] (95% CI)	P [a]	OR[b] (95% CI)	P [b]
FAT4	Nm	167 (43.5)	114 (30.8)	1.000		1.000		1.000	
	Pm	45 (11.7)	57 (15.4)	1.856 (1.174-2.932)	0.008*	1.751 (1.038-2.952)	0.036*	2.204 (1.168-4.159)	0.015*
	Hm	172 (44.8)	199 (53.8)	1.695 (1.239-2.319)	0.001*	1.418 (0.986-2.038)	0.060	1.434 (0.913-2.251)	0.117
	Tpm	217 (56.5)	256 (69.2)	1.728 (1.282-2.331)	<0.001*	1.486 (1.052-2.099)	0.024*	1.583 (1.031-2.430)	0.036*
SOX11	Nm	162 (42.1)	131 (36.4)	1.000		1.000		1.000	
	Pm	32 (8.3)	62 (17.2)	2.396 (1.475-3.891)	<0.001*	2.583 (1.467-4.546)	0.001*	2.530 (1.289-4.969)	0.007*
	Hm	191 (49.6)	167 (46.4)	1.081 (0.793-1.474)	0.621	0.947 (0.659-1.362)	0.770	0.945 (0.608-1.468)	0.800
	Tpm	223 (57.9)	229 (63.6)	1.270 (0.945-1.706)	0.112	1.159 (0.822-1.636)	0.400	1.155 (0.760-1.756)	0.499

Nm, negative methylation; Pm, positive methylation; Hm, heterogeneous methylation; Tpm, total positive methylation
CI, confidence interval; OR, odds ratio. *Statistically significant.
[a] Adjusted for BMI, occupation, family history of GC, age, gender.
[b] Adjusted for BMI, occupation, family history of GC, age, gender, regular diet, salt, green vegetables, freshwater fish, garlic, *H.pylori*, drinking, overnight food, fried food.

Table 3. Association between methylation status of genes and risk of GC by age.

Methylation status		< 60 years				≥ 60 years			
		OR (95% CI)	P	OR[a](95% CI)	P [a]	OR (95% CI)	P	OR[a] (95% CI)	P [a]
FAT4	Nm	1.000		1.000		1.000		1.000	
	Pm	1.849 (0.973-3.517)	0.061	1.548 (0.759-3.160)	0.230	1.866 (0.970-3.590)	0.062	1.950 (0.893-4.260)	0.094
	Hm	1.878 (1.223-2.886)	0.004*	1.280 (0.788-2.078)	0.318	1.513 (0.953-2.401)	0.079	1.586 (0.905-2.778)	0.107
	Tpm	1.872 (1.244-2.817)	0.003*	1.336 (0.845-2.112)	0.215	1.585 (1.020-2.463)	0.041*	1.661 (0.971-2.841)	0.064
SOX11	Nm	1.000		1.000		1.000		1.000	
	Pm	1.986 (0.987-3.997)	0.054	2.105 (0.946-4.683)	0.068	3.038 (1.540-5.993)	0.001*	3.342 (1.480-7.545)	0.004*
	Hm	0.956 (0.626-1.458)	0.833	0.725 (0.446-1.180)	0.196	1.291 (0.814-2.046)	0.278	1.405 (0.799-2.468)	0.237
	Tpm	1.092 (0.730-1.635)	0.668	0.881 (0.555-1.398)	0.591	1.561 (1.008-2.417)	0.046*	1.721 (1.008-2.936)	0.047*

Nm, negative methylation; Pm, positive methylation; Hm, heterogeneous methylation; Tpm, total positive methylation
CI, confidence interval; OR, odds ratio. *Statistically significant.
[a]Adjusted for BMI, occupation, family history of GC, gender.

Table 4. Association between methylation status of genes and risk of GC by *H.pylori* infection.

Methylation status		Negative *H. pylori* infection				Positive *H. pylori* infection			
		OR (95% CI)	P	OR^a(95% CI)	P^a	OR (95% CI)	P	OR^a (95% CI)	P^a
FAT4	Nm	1.000		1.000		1.000		1.000	
	Pm	1.824 (0.951-3.497)	0.071	1.781 (0.821-3.861)	0.144	1.879 (0.979-3.607)	0.058	1.714 (0.819-3.584)	0.152
	Hm	1.540 (0.974-2.435)	0.065	1.168 (0.674-2.026)	0.580	1.762 (1.138-2.728)	0.011*	1.522 (0.922-2.513)	0.101
	Tpm	1.602 (1.038-2.471)	0.033*	1.295 (0.773-2.169)	0.326	1.785 (1.174-2.713)	0.007*	1.560 (0.966-2.521)	0.069
SOX11	Nm	1.000		1.000		1.000		1.000	
	Pm	2.537 (1.237-5.202)	0.011*	3.323 (1.385-7.973)	0.007*	2.255 (1.150-4.425)	0.018*	2.284 (1.053-4.952)	0.036*
	Hm	1.342 (0.846-2.129)	0.212	1.142 (0.648-2.011)	0.647	0.916 (0.597-1.406)	0.689	0.834 (0.510-1.365)	0.470
	Tpm	1.513 (0.973-2.352)	0.066	1.409 (0.823-2.411)	0.211	1.109 (0.739-1.666)	0.617	1.023 (0.641-1.634)	0.924

Nm, negative methylation; Pm, positive methylation; Hm, heterogeneous methylation; Tpm, total positive methylation

CI, confidence interval; OR, odds ratio. *Statistically significant.

^a Adjusted for BMI, occupation, family history of GC, age, gender

Interactions between *FAT4*, *SOX11* methylation and environmental factors on the risk of GC

According to the results of gene-environment interactions analyses, the significant interaction between *FAT4* methylation and increased consumption of freshwater fish (≥ 1 times/week) displayed an antagonistic effect on the risk of GC (OR = 0.328, 95% CI: 0.142-0.762, P = 0.009). *FAT4* methylation significantly increased risk of GC at high consumption of freshwater fish (≥ 1 times/week) and relatively slight increased risk at low consumption (< 1 times/week; OR = 7.446, 95% CI: 4.143-13.380 and OR = 2.215, 95% CI: 1.423-3.448, respectively; Table S5). As for *SOX11* methylation, a marginally significant antagonistic interaction with high salt intake was observed (OR = 0.490, 95% CI: 0.242-0.995, P = 0.048; Table S6).

Discussion

Aberrant DNA methylation, which leads to inappropriate silencing of tumor suppressor and other cancer-related genes in cancer cells, is the most well-defined epigenetic change in the initiation and progression of GC [5]. Two considered potential tumor suppressor genes *FAT4* and *SOX11* involved in this study have been shown to participate in a series of cancer related molecular events. *FAT4* exerts a tumor suppressor role in GC cell lines by modulating Wnt/β-catenin signaling [15]. Aberrant *SOX11* promoter methylation has been demonstrated to be associated with poor prognosis in GC [17]. Results in this research indicated that compared with Nm, subjects with *FAT4* Pm and Tpm exhibited 2.204-fold and 1.583-fold increased risk of GC, respectively. *SOX11* Pm associated with the risk of GC (OR = 2.530), while Tpm and Hm had no association with GC risk. Inconsistent with our findings, Tomomitsu Tahara *et al.* failed to find any association between *SOX11* methylation in blood-derived DNA and GC risk [25], perhaps due to relative small sample size (72 GC patients and 69 controls) and different detection methods.

It has been uncovered that aging can alter DNA methylation, and *H. pylori* infection can trigger inflammatory response and then also induce accumulated aberrant DNA methylation [22, 29]. Based on above mentioned points, we performed stratified analyses by age and *H. pylori* infection. The result of age-stratified analysis indicated that individuals with *SOX11* methylation had a higher risk of GC only in elder, which might help explain the late onset of GC. Moreover, significant associations between *SOX11* Pm and GC risk retained in both the *H. pylori* positive and negative subjects.

Considerable evidence indicated that methylation changes in cancer patients occur systematically and can be measured in surrogate nontumor tissues [26, 30]. Different cancers elicit a clearly recognizable immune response in peripheral blood [31]. The differences in blood-derived DNA methylation in cancer cases can be largely explained by systematic differences in the methylation characteristics of the leukocyte subsets [32]. However, the exact mechanism underlying the alterations in the methylation of peripheral blood-derived DNA among GC-sensitive individuals remains unclear. It is also worth noting that since the negligible density, circulating tumor cell DNA is unlikely to interfere with the results of blood-derived DNA methylation [33-35]. Several previous studies have demonstrated that DNA methylation changes in peripheral blood leukocytes is a detectable biological indicator for GC risk assessment [35-37]. Our research once again verified that blood-derived DNA methylation changes can be detected. Furthermore, compared with cancer biopsy, the detection of peripheral blood leukocytes DNA methylation has the advantages of noninvasively and relatively inexpensive.

Heterogeneous methylation refers to multiple epialleles, each with a different pattern of methylated and unmethylated CpG sites, can co-exist for a given region [38]. Many previous studies have reported that heterogeneous methylation could occur in different cancers [39-41]. However, fewer researches explored it in a relative large sample for GC. Our data showed

that both *FAT4* and *SOX11* had heterogeneous methylation in GC patients and cancer-free controls. *FAT4* Hm affected 53.8% of GC patients, significant higher than 44.8% of cancer-free controls, while no significant association between *SOX11* Hm and GC risk was found. Speculative comments advanced by Azhikina *et al.* that heterogeneous methylation may disturb transcription process but not be sufficient to abolish transcription of the gene could partly explain these results [39]. Nevertheless, the physiological and clinical significance of heterogeneous methylation is still debated and need to be further researched.

MS-HRM was used in this research to assess *FAT4* and *SOX11* methylation status. It is a fast, cost- and labor-efficient technology that allowed us to quantify methylation or recognize heterogeneous methylation in a large sample panel [39, 42]. It is noteworthy that heterogeneous methylation can only be qualitative through MS-HRM, quantifying the levels of heterogeneous methylation usually need sequencing methodologies [38]. However, due to the relatively high cost of pyrosequencing, it is unsuitable for this large sample size research.

In this study, univariate and multivariate logistic regression analysis showed that freshwater fish was a risk factor for GC, which was somewhat unexpected. Several researches have reported that freshwater fish raised in contaminated water may have a relatively high level of toxic chemicals that could increase susceptibility to cancer [43-45], which to some extent explained our unexpected result. Besides, an antagonistic interaction between *FAT4* methylation and increased consumption of freshwater fish was also observed. Freshwater fish is rich in methionine, which serves as the substrate for S-adenosyl methionine (SAM) and plays a critical role in maintaining the flux of methyl groups for remethylation [46]. Long-term low dietary methionine intake can disturb methionine cycle and then induce normally non-methylated DNA situation to be aberrantly hypermethylated [47], which could provide a biologically plausible basis to *FAT4*-fish interactions. Furthermore, analyses of the relationship between *FAT4* methylation and environmental factors in all subjects showed that freshwater fish could decrease the risk of *FAT4* methylation, which might further help account for this interaction.

High salt intake was found to be another GC risk factor in this study. In addition, *SOX11* methylation interacted with high salt intake displayed an antagonistic effect on GC risk was also observed. Although no significant association between *SOX11* methylation and high salt intake existed in all subjects, negative association was observed in cases (P = 0.043; data not shown), which might provide another way to support the observed antagonistic interaction. Environmental factors may influence tumorigenesis by affecting the process of DNA methylation of certain key genes [48, 49]. Making changes in environmental exposures might provide an opportunity to inhibit or reverse this process and then counteract cancer [50]. However, the potential biological mechanisms underlying environmental factors interfering with DNA methylation in the initiation and progression of GC are complex and variable, further explorations are needed.

Additionally, gene polymorphisms also play an important role in the occurrence of GC [51, 52]. Previous exome sequencing identified a greater than expected number of non-synonymous mutations of *FAT4* in GC [53], but no studies have reported the associations between the polymorphisms of *SOX11* and GC risk. Moreover, gene polymorphisms could also interacted with gene methylation status to affect the occurrence and development of GC [54, 55]. Further studies are needed to investigate the relationship between *FAT4*, *SOX11* gene polymorphisms and GC risk, as well as the interactions between methylation and polymorphisms of these two genes on the risk of GC.

There are two main limitations in this case-control study. First, recall bias might still be inevitable in the process of collecting information on environmental factors, although measures were taken to control this bias. Second, the observed methylation changes in GC patients occurred before or after tumorigenesis remain unclear.

In conclusion, this study suggested that *FAT4* methylation in peripheral blood leukocytes was associated with the risk of GC, while *SOX11* was controversial and needs to be more investigated. Gene methylation status interacted with environmental factors might influence GC susceptibility.

Abbreviations

GC: gastric cancer; *FAT4*: FAT atypical cadherin 4; *SOX11*: SPY-Box11; MS-HRM: Methylation-sensitive high resolution melting; *H. pylori*: *Helicobacter pylori*; ELISA: enzyme-linked immuno-sorbent assay; BMI: body mass index; OR: odds ratio; CI: confidence interval; SAM: S-adenosyl-methionine; Nm: negative methylation; Pm: positive methylation; Hm: heterogeneous methylation; Tpm: total positive methylation.

Supplementary Material

Supplementary tables.
http://www.jcancer.org/v09p2275s1.pdf

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Human Research and Ethics Committee of Harbin Medical University.

Competing Interests

The authors have declared that no competing interest exists.

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