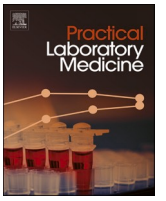
Practical Laboratory Medicine 40 (2024) e00406

Contents lists available at ScienceDirect

Practical Laboratory Medicine

journal homepage: www.elsevier.com/locate/plabm



Registered Report Stage II

Detection of CDH1 gene promoter hypermethylation in gastric cancer and chronic gastritis

Mitra Bayat [a], Amir Shirgir [a], Arash Kazemi Veisari [b], Rouhallah Najjar Sadeghi [a,c,*]

[a] Faculty of Medicine, Department of Clinical Biochemistry, Mazandaran University of Medical Sciences, Sari, Iran
[b] Gut and Liver Research Center, Non-Communicable Disease Research Institute, Mazandaran University of Medical Sciences, Sari, Iran
[c] Molecular and Cell Biology Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

ARTICLE INFO

Keywords:
CDH1
E-cadherin
Hypermethylation
Gastric cancer
Chronic gastritis
H. pylori infection
Depth of invasion
Nodal invasion

ABSTRACT

Aim: The current study aimed to assess the frequency of *CDH1* promoter gene hypermethylation in gastric cancer and chronic gastritis and its correlation with clinicopathological aspects.
Methods: Methylation-specific PCR was used to detect CDH1 promoter gene hypermethylation in 53 chronic gastritis patients and 40 gastric cancer patients along with normal adjacent tissues.
Results: The chronic gastritis group comprised 29 males and 24 females with a mean age of 51.8 ± 12.96 years, and 49.1 % of them were positive for H. pylori infection. The frequency of *CDH1* hypermethylation in gastritis lesions was 18.8 %. *CDH1* hypermethylation showed a significant correlation with H. pylori infection ($p = 0.039$), but no significant association was observed with other clinical features. The gastric cancer group consisted of individuals with a mean age of 65.4 ± 10.6, among them, 77.5 % were male and 22.5 % were female, 62.5 % had PT3 tumors, 40 % had PN1 lymph node involvement, and the majority (47.5 %) of samples were obtained from body segment. *CDH1* hypermethylation was significantly associated with depth of invasion ($p = 0.017$) and nodal invasion ($p = 0.041$) in this group. In both groups, normal adjacent specimens lacked CDH1 hypermethylation, and there was no statistically significant correlation between CDH1 hypermethylation and age at which the tumor was diagnosed, gender, activity level, or tumor location.
Conclusion: This study demonstrates that E-cadherin methylation is associated with some characteristics of chronic gastritis and gastric cancer. These findings support previous research indicating that CDH1 hypermethylation may play a significant role in the development of gastric cancer.

1. Introduction

Gastric carcinoma (GC) is one of the most prevalent types of cancer in the world, characterized by a poor prognosis and a 5-year survival rate for Iranian patients that is below 25 % [1]. GC is also one of the five most common types of cancer in the Iranian population [2], ranking first in males and third in females [3]. As a result of the absence of specific symptoms and the subsequent detection of the disease at an advanced stage, GC stands as the leading cause of cancer-related mortality for individuals of all genders in Iran [4].

* Corresponding author. Faculty of Medicine, Department of Clinical Biochemistry, Mazandaran University of Medical Sciences, Sari, Iran.
E-mail addresses: r.nsadeghi@mazums.ac.ir, najjarsadeghi@yahoo.com (R. Najjar Sadeghi).

https://doi.org/10.1016/j.plabm.2024.e00406
Received 14 April 2024; Received in revised form 21 May 2024; Accepted 22 May 2024
Available online 23 May 2024

The intestinal form of GC develops in a sequential manner, beginning with normal gastric mucosa and progressing through chronic gastritis, multifocal atrophic gastritis, intestinal metaplasia, dysplasia, and invasive adenocarcinoma, according to a model developed by Pelayo and Correa [5]. Chronic gastritis is still a relatively common disease and it is estimated that estimate that more than half of the world population is believed to have this disease to some degree and extent [6].

The precise molecular mechanism through which certain gastritis lesions advance to the end stage and lethal final, while others do not, remains poorly understood, despite the fact that the fundamental contours of GC carcinogenesis are widely recognized. These ambiguities make it necessary to identify the molecular mechanisms underlying the development of GC and the identification of novel molecular biomarkers for early detection at the level of precancerous GC lesions.

Epigenetic modifications are considered to be among the earliest, most comprehensive, and most common genomic alterations occurring during carcinogenesis [7]. Global genome hypomethylation occurs predominantly at repetitive elements and promotes genomic instability, unwanted activation of transposable elements, and cancer progression. In this context, regional hypermethylation of promoter CpG islands leads to the silencing of tumor suppressor genes, hence contributing to GC [8]. Accumulating evidence indicates that DNA hypermethylation and other epigenetic aberrations are involved in the development of GC [9]. In addition, several DNA methylation-based patterns of GC have been reported [10].

Mutations in the gene encoding the Ca^{2+-}dependent cell adhesion molecule, E-cadherin (*CDH1*) have been linked to carcinogenesis [10,11]. E-cadherin is an essential component of the cytoskeleton and cell adhesion network; consequently, loss of its function or reduced expression results in decreased cellular adhesion and increased tumor cell invasion [12].

Previous studies revealed that *CDH1* methylation and consequent loss of the gene expression have important roles in the origin and development of some tumors, such as prostate carcinoma [13], non-small cell lung carcinoma [14], liver carcinoma [15], esophageal cancer [16], and breast cancer [17].

CDH1 is frequently hypermethylated in GC, with a prevalence ranging from 26.2 % to 84 %. However, most of these studies did not clearly specify the histopathologic type of the samples [18–22]. Only two studies explicitly mentioned that the samples were intestinal-type adenocarcinomas, and in these cases, the frequency of CDH1 methylation was approximately 53.3 % to 58 % [23,24].

While CDH1 methylation in the GC was frequently evaluated, precancerous lesions of the GC received little attention. The occurrence of methylation in intestinal metaplasia and chronic gastritis has been documented in a limited number of studies as 36.1 % and 57 %, respectively [24,25].

In order to determine the methylation status of the E-cadherin promoter in malignant and precancerous gastric epithelium, we monitored the methylation profile of the CDH1 gene promoter using a methylation-specific PCR (MSP) assay.

2. Materials and methods

2.1. Patients and tissue samples

Prior to specimen collection, written informed consent was obtained from all subjects, and the Ethics Committee of Mazandaran University of Medical Sciences approved the procedures of the study. A total of 53 chronic gastritis samples and 40 primary GC samples with normal adjacent tissues were collected from patients undergoing upper GI endoscopy at the Tuba Endoscopy ward in Sari, Iran. None of the patients had pre-operative chemotherapy or radiotherapy. In order to categorize patients with chronic gastritis, H&E was performed to detect the presence of intestinal dysplasia and intestinal metaplasia. Infection with *H. pylori* was assessed by histological examinations. The histological subtypes of GC samples were classified according to the Lauren criteria [3].

2.2. Genomic DNA extraction

Genomic DNA from all specimens was extracted using the QIAamp® DNA Mini Kit (Qiagen, GmbH, Germany) according to the manufacturer's instructions. The quality and quantity of the extracted DNA were assessed by the A260/280 ratio and 260 nm using a WPA spectrophotometer (Biochrom WPA Biowave II UV/Visible), respectively. DNA integrity was also checked by agarose gel electrophoresis.

2.3. DNA treatment by sodium bisulfite

The extracted DNA underwent bisulfite conversion *via* the EpiTect® DNA Mini Kit (Qiagen, GmbH, Germany) according to the manufacturer's instructions. Afterward, bisulfite-converted DNA was used immediately or stored at −20 °C for subsequent methylation analysis.

2.4. Methylation-specific polymerase chain reaction

Methylation-specific polymerase chain reaction (MSP) was used to distinguish methylated from unmethylated DNA by applying two sets of primers: one specific for the methylated allele and another for the unmethylated one [26]. PCR was performed using controls for unmethylated and methylated alleles, which were constructed from CpG-methylated HeLa genomic DNA (New England Biolabs®) and DNA extracted from normal human blood, respectively.

The amplification process was carried out in a thermal cycler (Applied Biosystem) with the primers displayed in Table 1, and the cycling program was as follows: initial denaturing at 95 °C for 15 min (one cycle), followed by 30 cycles of 95 °C for 30 s, 64.5 °C for 30

s, and at 72 °C for 35 s, and a final extension step at 72 °C for 10 min in each run. The PCR reaction mixture included TEMPase Hot Start Master Mix (Ampliqon, Denmark) with 1.5 mM $MgCl_2$. The PCR products were then visualized on a 2 % agarose gel stained with GelRed.

2.5. Statistical analysis

SPSS software version 18 was used for the analysis of the obtained data. Two-sided Fisher's exact test and χ^2 tests were applied to detect a probable association between CDH1 methylation and categorized clinico-pathological parameters. To compare quantitative data from different groups, the independent T-test was utilized. The normality of continuous data was examined using the Kolmogorov-Smirnov test. P-values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Patients

In total, 53 histologically confirmed samples from chronic gastritis and 40 from cancerous lesions were included in this study (Table 2). Based on the Kolmogorov-Smirnov test, the distribution of age data was normal in both gastritis and gastric cancer groups (p-values 0.077 and 0.17, respectively).

The demographic characteristics of patients revealed that the gastritis group consisted of 29 males and 24 females, while the cancer group comprised of 31 males and 9 females (see Table 2). Among chronic gastritis patients, the majority of tissue specimens were located in the antrum (90.6 %), showed no signs of intestinal metaplasia (71.7 %), and negative for *H. pylori* (50.9 %). Additionally, these specimens exhibited mild activity (64.2 %), as shown in Table 3.

Conversely, a significant portion **(**47.5 %**)** of the gastric cancer samples were derived from the body segment. Within this group, tumor cell infiltration into the subserosa layer (Pt3) and lymph nodes (Pn1-Pn3) was observed in 62.5 % and 85 % of the cases, respectively. In addition, vascular invasion was identified in 47.5 percent of the cancer patients, as shown in Table 4.

3.2. Association between clinicopathological features and DNA methylation

As shown in Figs. 1 and 2, the hypermethylation frequency of the E-cadherin gene promoter was 18.87 % in gastritis lesions and 32.5 % in gastric cancer. Notably, none of the normal adjacent samples showed hypermethylation. It was found that the frequency of methylation at the gene promoter of E-cadherin increases along Correa's cascade, from gastritis toward GC, but such a difference was not statistically significant (p-value = 0.1314, $\chi 2$ test).

Gender and age at diagnosis did not demonstrate a statistically significant difference between the two groups of patients in the presence or absence of E-cadherin hypermethylation. A correlation between *H. pylori* infection and E-cadherin promoter hypermethylation was identified, and this correlation was statistically significant (Fisher's exact test: two-tailed p-value = 0.039). While all methylated samples were taken from the antrum, there was no statistically significant association between methylation and the location of tumors (Table 5).

In gastric cancer group, a significant association was observed between CDH1 hypermethylation and both the depth of invasion and lymph node invasion.

E-cadherin methylation was notably associated with distinct tumor characteristics. All tumors confined to the muscularis propria layer (T2) were found to be unmethylated, whereas in 12 out of 13 (92.3 %) methylated specimens, tumors were localized in the serosa (Pt3)**.** E-cadherin methylation also demonstrated an association with regional nodal metastasis, as 8 out of 13 methylated specimens (61.5 %) were observed in patients with the PN2 stage (Table 6). However, CDH1 hypermethylation remained independent of variables such as age, gender, vascular invasion, perineural invasion, tumor site, and size (Table 6).

4. Discussion

Gastric cancer ranks among the most common and lethal types of cancer, yet its molecular basis is not well understood. According to Correa's cascade of gastric carcinogenesis, chronic gastritis can progress to gastric cancer through stages including intestinal metaplasia and dysplasia. Consequently, it is crucial to trace molecular abnormalities from the initial stages to the advanced stages of the disease. This could offer valuable insights into the mechanisms of gastric cancer initiation, development, and metastasis.

In gastric carcinogenesis, aberrant promoter methylation plays a fundamental role through inactivating tumor suppressor genes

Table 1
List of primers used for detection methylation status of CDH1 by methylation-specific PCR.

		Sequence	Product length
Methylated	Forward	5′ GGTGGGCGGGTCGTTAGTTTCG 3′	**106 bp**
	Reverse	5′ AAAACACCGCCCCCCGTACCG 3′	
Unmethylated	Forward	5′ GTTGGGTGGGTGGGTTGTTAGTTTTG 3′	**113 bp**
	Reverse	5′ CCAAAAACACCACCCCCCATACCA 3′	

Table 2
Comparing the Basal demographic characteristics of patients with chronic gastritis and gastric cancer.

Character		Chronic gastritis	Gastric cancer	P value
Age (mean ± SD(	51.98 ± 12.96		65.4 ± 10.6	0.0001[a]
Age (Rang)		28–80	48–88	
Gender	Male	29 (54.7)	31 (77.5)	0.023[b]
	Female	24 (45.3)	9 (22.5)	
Hypermethylation	Methylated	10 (18.87)	13(32.5)	0.131 [b]
	Unmethylated	43 (81.13)	27 (67.5)	

t-test [a] and Chi-squared test [b] were used. Wherever it was not mentioned, the data were expressed as a number (%).

Table 3
Histopathologic characteristics and H. pylori infection of chronic gastritis samples according to updated Sydney classification.

	Character	Number (%)
Site of sampling	Antrum	48(90.6)
	Body	2(3.8)
	Cardia	3(5.7)
Intestinal metaplasia	Positive	15(28.3)
	Negative	38(71.7)
H.pylori	Positive	26(49.1)
	Negative	27(50.9)
Activity	Mild	34(64.2)
	Moderate	15(28.3)
	Severe	4(7.5)

Table 4
Distribution of histopathological features of samples from patients diagnosed with gastric cancer.

Character		Number (%)
Depth of invasion	Pt2	2 (5)
	Pt3	25(62.5)
	Pt4	13(32.5)
Lymph node invasion	PN0	5 (12.5)
	PN1	16(40)
	PN2	14(35)
	PN3	4 (10)
Vascular invasion	Yes	19(47.5)
	No	21(52.5)
Perineural invasion	Yes	25(62.5)
	No	15(37.5)
Site of sampling	Body	19(47.5)
	Cardia	10(25)
	Fundus	5 (12.5)
	Pylorus	6(15)

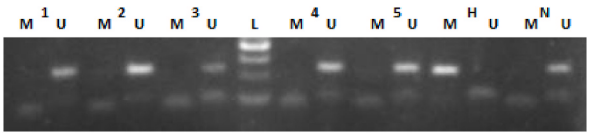


Fig. 1. Gel electrophoresis using MSP primers on normal tissues. L: size marker 50 bp, M: methylated, U: unmethylated, 1, 2, 3, 4, 5: normal tissues, H: CpG Methylated HeLa Genomic DNA, N: DNA extracted normal person's blood.

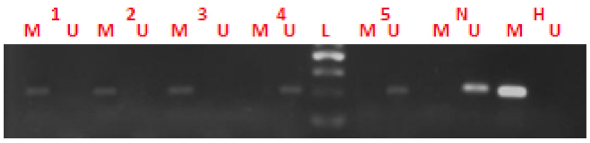


Fig. 2. Gel electrophoresis using MSP primers on gastric cancer tissues. L: size marker 50 bp, M: methylated, U: unmethylated, H: CpG Methylated HeLa Genomic DNA, N: DNA extracted normal person's blood.

Table 5
The correlation between Patients 'characteristics, and E-cadherin methylation status in chronic gastritis group.

		Methylated	Unmethylated	P value
Age (mean ± SD)		51 ± 11.72	52.21 ± 13.36	0.793[a]
Gender	Male	4 (13.7)	25 (86.2)	0.482 [b]
	Female	6 (25)	18 (75)	
H.P. Infection	Positive	8 (80)	18 (41.8)	**0.039** [b]
	Negative	2 (20)	25 (58.2)	
Intestinal metaplasia	Positive	4 (22.6)	11(73.4)	0.442 [b]
	Negative	6 (15.8)	32 (84.2)	
Activity	Mild	8 (23.5)	26 (76.5)	0.442 [b]
	Moderate	2 (13.3)	13 (86.7)	
	Severe	0	4 (100)	
Location	Antrum	10(20.8)	38 (79.2)	0.526[c]
	Body	0	2 (100)	
	Cardia	0	3 (100)	

t-test [a], Fischer exact test [b] and [c] $\chi 2$ test were used to find if there is a statistically significant difference between methylation status and characters. Data was shown as Number (%) and bold values denote statistical significance at the $p < 0.05$ level.

[27]. *CDH1* hypermethylation has been linked to reduced gene expression [17]. Therefore, its inactivation through promoter hypermethylation may play a determinative role in the development of human cancer, especially gastric cancer [9].

In accordance with previous studies [20,22,24,28], *CDH1* promoter hypermethylation was not associated with the age of diagnosis in cases of chronic gastritis and gastric cancer. This finding shows that *CDH1* hypermethylation is not age-dependent, and any methylation occurring in its promoter is the cause or effect of cancer-related processes. However, age-related methylation of *CDH1* was reported by Ben Ayed-Guerfali et al. [23]. This contradiction can be caused by two issues. First, the majority of age-related methylation takes place in exonic or far-upstream regions within CpG islands of promoters; however, a small region, which includes the transcription start site, remains unmethylated even within the same CGI [9]. Consequently, the region of interest for CDH1 in our research may have been distinct from that of Ben Ayed-Guerfali.

The second justification is the sampling from diverse and difference populations. While certain data suggests that boundaries exist at both termini of CpG islands to ensure unmethylation in normal tissue, these boundaries may be disregarded during carcinogenesis and tumor suppressor gene silencing [29]. The degree and type of this epigenetic revolutions are influenced by genetic susceptibility and environmental stimuli, such as *H. pylori* infection [30].

Consistent with previous studies, none of the normal specimens from gastritis and GC groups exhibited hypermethylation in *CDH1*. As a result, it can be inferred that epigenetic modifications of this gene probably did not play a role in the early stages of carcinogenesis.

Subsequently, we investigated the relationship between CDH1 hypermethylation and *H. pylori* infection in gastritis lesions, revealing a positive correlation between CDH1 methylation and *H. pylori* infection. This correlation indicates the undeniable role of *H. pylori*-related inflammation in inducing hypermethylation, which has clear implications for the gastric carcinogenesis process.

Prior studies have indicated that the number of methylated genes is higher in *H. pylori*-positive chronic gastritis than in the negative cases and ***H. pylori*** infection is significantly linked with the hypermethylation of certain genes, such as CDH1 [20,24]. Conversely, some evidence has shown that successful eradication of H. pylori can potentially reduce methylation density in exon 1 and the promoter region of E-cadherin hypermethylation [31]. This suggests that ***H. pylori*** infection may directly contribute to E-cadherin methylation and suppression, either independently or in conjunction with other collaborative mechanisms. Of note, Yu et al. failed to establish a relationship between ***H. pylori*** and E-cadherin hypermethylation [22]. Further research is warranted to clarify the precise mechanisms of interaction between H. pylori infection and E-cadherin hypermethylation.

The methylation frequencies of E-cadherin in gastric cancer samples were reported to be 32.5 %, which is lower than the ranges

Table 6
The correlation of CDH1 Hypermethylation with clinicopathological findings of gastric cancer.

		Methylated	Unmethylated	P value
Age (mean ± SD)		68.38 ± 9.38	63.92 ± 11.18	0.227[a]
Age	<65	5	14	0.248 [b]
	>66	8	11	
Gender	Male	11(33.6)	20 (.)	0.96 [b]
	Female	2 (22.2)	7	
Depth of invasion	Pt2	0	2 (100)	**0.017** [b]
	Pt3	12(48)	13 (52)	
	Pt4	1(7.7)	12 (92.3)	
Lymph node invasion	PN0	1(20)	4 (80)	**0.041** [b]
	PN1	2(14.3)	14 (85.7)	
	PN2	8(52.2)	6 (47.8)	
	PN3	2(50)	2 (50)	
Vascular invasion	Yes	7(36.8)	12 (63.2)	0.577 [b]
	No	6(28.6)	15 (71.4)	
Perineural invasion	Yes	8(32)	17 (68)	0.931 [b]
	No	5(33.3)	10 (72.7)	
Site of sampling	Body	8(42.1)	11 (57.9)	0.422 [b]
	Cardia	3(30)	7 (70)	
	Fundus	0	5 (100)	
	Pylorus	2(33.3)	4 (72.7)	
Size of greatest dimension (mean ± SD)		5.73 ± 1.57	6.42 ± 2.48	0.363[c]

t-test [a], Fischer exact test [b] and [c] $\chi 2$ test were used to find if there is a statistically significant difference between methylation status and characters. Data was shown as Number (%) and bold values denote statistical significance at the $p < 0.05$ level.

previously reported [23,24]. This discrepancy in methylation frequency can be attributed to various factors, such as the quality of DNA and next treatment, and PCR conditions. However, the selection of CpG islands and the design of the primers may be the most significant.

The current study observed a gradual rise in the percentage of CDH1 methylation across the stages of Correa's cascade, starting from normal tissues and progressing through chronic gastritis without intestinal metaplasia, chronic gastritis with intestinal metaplasia, and finally to gastric cancer samples. The percentages of CDH1 methylation in these stages were 0 %, 15 %, 22 %, and 32.5 %, respectively. Although this difference was not statistically significant, it suggests that CDH1 methylation likely contributes to the carcinogenesis of specific subtypes of gastric cancer. Therefore, it is necessary to conduct more investigations in larger groups that include all subtypes to confirm this finding.

Hypermethylation of CDH1 was found to be correlated with GC cases exhibiting depth of invasion and nodal metastasis, as indicated by prior research. Peritoneal metastasis is one of the most common forms of metastasis in gastric cancer [32]. Although the exact mechanism is unknown, hypermethylation and the resulting downregulation of CDH1 likely occur during the development of GC. This process enables tumor cells to easily traverse basement membranes and enter adjacent tissues and vessels. Furthermore, it facilitates both proximal and distal metastasis.

Depth of invasion and nodal metastasis may be associated with poor prognosis[33] and finding a relationship between these parameters and *CDH1* methylation may indicate the influencing role of *CDH1* methylation in the development of advanced stages of gastric cancer.

Consistent with prior researches, hypermethylation of CDH1 was found to be associated with higher depth of invasion and nodal metastasis [18,22,24]. It is worth noting that peritoneal metastasis has become the predominant mode of dissemination in gastric cancer cases [34]. The underlying mechanism for this observation remains unclear, but it has been postulated that during gastric cancer development, CDH1 hypermethylation and subsequent downregulation of E-cadherin may facilitate tumor cell infiltration through the basement membrane into adjacent tissues and vessels, promoting proximal and distal metastases. The depth of invasion and nodal metastasis are frequently indicative of a poor prognosis; therefore, establishing a correlation between these parameters and CDH1 hypermethylation may implies the potential impact of hypermethylation on the progression toward advanced stages of gastric cancer.

In accordance with previous reports, no correlation was identified between CDH1 methylation and other parameters, such as age, gender, and tumor size [18,22,24].

5. Conclusion

Overall, this study demonstrates an association between certain characteristics of chronic gastritis or gastric cancer and E-cadherin methylation. Our results support previous findings suggesting that CDH1 hypermethylation may have a significant impact on gastric cancer development, particularly in advanced stages.

Further investigations on large and diverse cohorts are essential to fully understanding the functional significance of CDH1 methylation in promoting the advancement of gastric cancer and evaluating its implications for clinical and therapeutic strategies.

Funding

This work was supported by the Mazandaran University of Medical Sciences.

CRediT authorship contribution statement

Mitra Bayat: Writing – review & editing, Writing – original draft, Investigation. **Amir Shirgir:** Writing – original draft, Investigation. **Arash Kazemi Veisari:** Writing – original draft, Resources, Data curation. **Rouhallah Najjar Sadeghi:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Data availability

The data that has been used is confidential.

Acknowledgments

This work was supported by the Mazandaran University of Medical Sciences, Sari, Iran. We would like to thank the lab staff of the Tuba Endoscopy ward for their kindness and support.

References

[1] K. Kalan Farmanfarma, N. Mahdavifar, S. Hassanipour, et al., Epidemiologic study of gastric cancer in Iran: a systematic review, Clin. Exp. Gastroenterol. 13 (2020) 511–542.
[2] T. Dehdari, L. Dehdari, S. Jazayeri, Diet-related stomach cancer behavior among Iranian college students: a text messaging intervention, Asian Pac J Cancer Prev 17 (12) (2016) 5165–5172.
[3] G. Roshandel, A. Ghanbari-Motlagh, E. Partovipour, et al., Cancer incidence in Iran in 2014: results of the Iranian national population-based cancer registry, Cancer Epidemiol 61 (2019) 50–58.
[4] A. Akhavan, F. Binesh, A. Seifaddiny, Results of combination chemotherapy and radiation therapy in non-metastatic gastric cancer in Yazd–Iran, Indian J. Cancer 52 (1) (2015) 40–43.
[5] P. Correa, M.B. Piazuelo, The gastric precancerous cascade, J Dig Dis 13 (1) (2012) 2–9.
[6] P. Sipponen, H.I. Maaroos, Chronic gastritis, Scand. J. Gastroenterol. 50 (6) (2015) 657–667.
[7] A. Nishiyama, M. Nakanishi, Navigating the DNA methylation landscape of cancer, Trends Genet. 37 (11) (2021) 1012–1027.
[8] G. Usui, K. Matsusaka, Y. Mano, et al., DNA methylation and genetic aberrations in gastric cancer, Digestion 102 (1) (2021) 25–32.
[9] Y. Qu, S. Dang, P. Hou, Gene methylation in gastric cancer, Clin. Chim. Acta 424 (2013) 53–65.
[10] M. Li, P. Zhang, The function of APC/CCdh1 in cell cycle and beyond, Cell Div. 4 (2009) 2.
[11] J. Masterson, S. O'Dea, Posttranslational truncation of E-cadherin and significance for tumour progression. Cells, tissues, organs 185 (1–3) (2007) 175–179.
[12] E.R. Fearon, Cancer: context is key for E-cadherin in invasion and metastasis, Curr. Biol. 29 (21) (2019) R1140–r1142.
[13] M. Sugiura, H. Sato, M. Kanesaka, et al., Epigenetic modifications in prostate cancer, Int. J. Urol. 28 (2) (2021) 140–149.
[14] K. Krishnamurthy, T.K. Mishra, A. Saxena, et al., Evaluating NISCH and CDH1 promoter hypermethylation in nonsmokers, cancer free smokers and lung cancer patients: a case control study, Indian J. Clin. Biochem. 34 (4) (2019) 458–464.
[15] X. Fan, S. Jin, Y. Li, et al., Genetic and epigenetic regulation of E-cadherin signaling in human hepatocellular carcinoma, Cancer Manag. Res. 11 (2019) 8947–8963.
[16] Z.Q. Ling, P. Li, M.H. Ge, et al., Hypermethylation-modulated down-regulation of CDH1 expression contributes to the progression of esophageal cancer, Int. J. Mol. Med. 27 (5) (2011) 625–635.
[17] J. Liu, X. Sun, S. Qin, et al., CDH1 promoter methylation correlates with decreased gene expression and poor prognosis in patients with breast cancer, Oncol. Lett. 11 (4) (2016) 2635–2643.
[18] N. Oue, Y. Mitani, J. Motoshita, et al., Accumulation of DNA methylation is associated with tumor stage in gastric cancer, Cancer 106 (6) (2006) 1250–1259.
[19] H. Suzuki, F. Itoh, M. Toyota, et al., Distinct methylation pattern and microsatellite instability in sporadic gastric cancer, Int. J. Cancer 83 (3) (1999) 309–313.
[20] T. Tahara, T. Shibata, M. Nakamura, et al., Increased number of CpG island hypermethylation in tumor suppressor genes of non-neoplastic gastric mucosa correlates with higher risk of gastric cancer, Digestion 82 (1) (2010) 27–36.
[21] T. Waki, G. Tamura, T. Tsuchiya, et al., Promoter methylation status of E-cadherin, hMLH1, and p16 genes in nonneoplastic gastric epithelia, Am. J. Pathol. 161 (2) (2002) 399–403.

[22] Q.M. Yu, X.B. Wang, J. Luo, et al., CDH1 methylation in preoperative peritoneal washes is an independent prognostic factor for gastric cancer, J. Surg. Oncol. 106 (6) (2012) 765–771.
[23] D. Ben Ayed-Guerfali, K. Benhaj, A. Khabir, et al., Hypermethylation of tumor-related genes in Tunisian patients with gastric carcinoma: clinical and biological significance, J. Surg. Oncol. 103 (7) (2011) 687–694.
[24] A.O. Chan, S.K. Lam, B.C. Wong, et al., Promoter methylation of E-cadherin gene in gastric mucosa associated with Helicobacter pylori infection and in gastric cancer, Gut 52 (4) (2003) 502–506.
[25] T. Tahara, T. Arisawa, T. Shibata, et al., Increased number of methylated CpG islands correlates with Helicobacter pylori infection, histological and serological severity of chronic gastritis, Eur. J. Gastroenterol. Hepatol. 21 (6) (2009) 613–619.
[26] J.G. Herman, J.R. Graff, S. Myöhänen, et al., Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands, Proc Natl Acad Sci U S A 93 (18) (1996) 9821–9826.
[27] K. Matsusaka, S. Funata, M. Fukayama, et al., DNA methylation in gastric cancer, related to Helicobacter pylori and Epstein-Barr virus, World J. Gastroenterol. 20 (14) (2014) 3916–3926.
[28] T.-L. Lee, W.K. Leung, M.W. Chan, et al., Detection of gene promoter hypermethylation in the tumor and serum of patients with gastric carcinoma, Clin. Cancer Res. 8 (6) (2002) 1761–1766.
[29] J.R. Graff, J.G. Herman, S. Myöhänen, et al., Mapping patterns of CpG island methylation in normal and neoplastic cells implicates both upstream and downstream regions in de novo methylation, J. Biol. Chem. 272 (35) (1997) 22322–22329.
[30] T. Kubota, K. Miyake, T. Hirasawa, Epigenetic understanding of gene-environment interactions in psychiatric disorders: a new concept of clinical genetics, Clin. Epigenet. 4 (1) (2012) 1.
[31] W.K. Leung, E.P. Man, J. Yu, et al., Effects of Helicobacter pylori eradication on methylation status of E-cadherin gene in noncancerous stomach, Clin. Cancer Res. 12 (10) (2006) 3216–3221.
[32] M. Acs, P. Piso, G. Glockzin, Peritoneal metastatic gastric cancer: local treatment options and recommendations, Curr. Oncol. 31 (3) (2024) 1445–1459.
[33] F. Li, Z. Chen, B. Tan, et al., Influential factors and prognostic analysis of blood vessel invasion in advanced gastric cancer, Pathol. Res. Pract. 216 (3) (2020) 152727.
[34] X. Kang, W. Li, W. Liu, et al., LIMK1 promotes peritoneal metastasis of gastric cancer and is a therapeutic target, Oncogene 40 (19) (2021) 3422–3433.