Examination of Whole Blood DNA Methylation as a Potential Risk Marker for Gastric Cancer

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Abstract

Whole blood DNA methylation analysis has been proposed to be a risk marker for cancer that can be used to target patients for preventive interventions. To test this, we examined whole blood DNA methylation of 16 CpG island promoters and LINE1 repetitive element in patients with gastric cancer and control subjects. Bisulfite pyrosequencing was used to quantify the methylation of 14 CpG island promoters (MINT25, RORA, GDNF, CDH1, RARAB2, ER, CDH13, MYOD1, SFRP1, P2RX7, SLC16A12, IGF2, DPYS, and N33) and LINE1 from 72 patients with gastric cancer, 67 control, and 52 healthy young individuals. Quantitative methylation-specific real-time PCR was also conducted for 3 CpG island promoters (MINT25, MYO3A, and SOX11). Among all sites tested, only a marginal increase in the methylation of the SFRP1 promoter was observed in the blood of patients with gastric cancer when compared with the control group (11.3% vs 10.5%; age-adjusted \( P = 0.009 \)), and this association was also seen in a validation set of 91 patients with gastric cancer (11.5% vs 10.5%; age-adjusted \( P = 0.001 \)). The methylation of 9 sites (GDNF, CDH1, RARAB2, CDH13, MYOD1, SFRP1, SLC16A12, DPYS, N33, and LINE1) and their mean \( Z \) score was correlated with higher age (\( R = 0.41, P < 0.0001 \)) and marginally with telomere shortening (\( R = -0.18, P = 0.01 \)) but not with gastric cancer risk (other than SFRP1 methylation). Variability in whole blood DNA methylation of cancer markers is primarily associated with aging, reflecting turnover of white blood cells, and has no direct link to gastric cancer predisposition. SFRP1 methylation in whole blood may be associated with gastric cancer risk. Cancer Prev Res; 6(10): 1093–100. ©2013 AACR.

Introduction

Gastric cancer is a major cause of cancer-related death, worldwide. *Helicobacter pylori* (*H. pylori*) plays an important role in gastric carcinogenesis, although the majority of individuals with *H. pylori* infection do not develop gastric cancer (1). Surveillance of these high-risk patients using reliable and accurate predictive markers is important for reducing the incidence of cancer and its mortality. It is now widely accepted that changes in DNA methylation patterns, particularly promoter hypermethylation and global (genome-wide) hypomethylation, contribute to cancer development and tumor growth (2, 3). Neoplastic growth is frequently preceded by aberrant promoter methylation, which leads to a loss-of-function for the genes that promote cell proliferation (4). Hypomethylation is thought to contribute to carcinogenesis by inducing genomic instability (5, 6), leading to the formation of abnormal chromosomal structures (7, 8). Gene promoter hypermethylation and global hypomethylation in tumor tissues are common events in the development of many types of cancer (4), whereas gene promoter hypermethylation and global hypomethylation are also observed in aged or inflamed tissues and are associated with cancer occurrence in their targeted tissues (9–11).

The DNA methylation status of various tissues has been shown to be associated with aging and perhaps also exposures encountered throughout life (12, 13), and therefore is now increasingly seen as a mechanism of cancer predisposition (14–17). Evaluating whole blood DNA methylation as a risk marker for cancer is of particular interest because peripheral blood DNA is a convenient tissue to assay for constitutional methylation, as its collection is noninvasive. It is possible that the methylation status of cancer target tissues (i.e., neoplastic cells and the surrounding tissue/field defect) might reflect acquired or inherited somatic events that are detectable in nontargeted tissues (methylation memory of exposures/inheritance) and correlate with cancer susceptibility. Thus, epigenetic signatures in whole blood DNA could reflect the interaction of host genetic and environmental factors associated with cancer susceptibility.
In addition, rare cases of constitutional DNA methylation of tumor suppressor genes have been reported and proposed to be predisposing to cancer. To evaluate this concept in gastric cancer, we investigated the methylation status of 16 CpG island promoters selected on the basis of either cancer-associated or age-related methylation, and also studied the LINE1 repetitive element (representative of global methylation) in whole blood DNA in patients with gastric cancer and control subjects. We also investigated the association between DNA methylation status and telomere length shortening, which is an indicator for cell turnover and aging (18, 19).

Materials and Methods

Samples analyzed

For screening, we used DNA from 8 primary gastric cancer tissues and 6 non-neoplastic gastric mucosae from healthy subjects. For testing, we use two different gastric cancer cohorts (training set: n = 72; validation set: n = 91) and 67 cancer-free subjects (controls) who were attending the endoscopy center of Fujita Health University (Toyoake, Aichi, Japan) from January 2005 to March 2010. Five milliliters of whole blood DNA was collected from each participant in an EDTA tube and stored frozen until DNA extraction. Whole blood DNA extraction was carried out using a commercial kit (FlexiGene DNA Kit, QIAGEN) and stored until processing for analysis. All patients with gastric cancer were admitted to Fujita Health University hospital for the treatment of gastric cancer. Noncancer patients underwent upper gastroscopy for various reasons, including yearly screening for gastric cancer, a secondary complete check-up after barium radiographic examination due to a suspicion of gastric cancer or peptic ulcer disease, and complaints of abdominal discomfort. They were finally diagnosed as not having gastric cancer. To avoid confounders, we excluded patients with chronic illness from the study. We also collected whole blood DNA from 52 healthy young individuals recruited from Japanese medical students and staff of the Fujita Health University School of Medicine from April 2006 to October 2007. The Ethics Committee of the Fujita Health University School of Medicine approved the protocol and written informed consent was obtained from all subjects.

Selection of candidate panels and CpG methylation analysis by bisulfite pyrosequencing and qMSP

The selection of genes is based on the hypothesis that the methylation status of targeted tissues (i.e., cancer and surrounding tissue) might reflect acquired or inherited somatic events that are detectable in nontargeted tissues (methylation memory of exposures/inheritance) and correlate with cancer susceptibility. We reasoned that there might be two approaches to selecting genes (all selected from the literature), one based on frequency of methylation in cancer (MINT25, RORA, GDNF, RARAB2, SLC16A12, SOX11, and MYO3A) and a separate one based on methylation in normal inflamed/aged tissues (CDH1, ER, CDH13, MYOD1, SFRP1, P2RX7, IGF2, and N33; refs. 9, 10, 20–26). Bisulfite-treated genomic DNA was used to evaluate the methylation status of these CpG island promoters by bisulfite pyrosequencing. We also evaluated the methylation status of the LINE1 repetitive element using bisulfite pyrosequencing. Bisulfite treatment of DNA was carried out with an Epitect Bisulfite Kit (Qiagen) according to the manufacturer’s protocol. Pyrosequencing was carried out using a Pyro-Gold Reagent Kit (Qiagen) and the results were analyzed using PyroMark Q96 ID software version 1.0 (QIAGEN). The primers used for pyrosequencing are listed in Supplementary Table S1. All bisulfite pyrosequencing was conducted at least twice and averaged. To increase sensitivity, for 3 genes (MINT25, SOX11, and MYO3A), we conducted quantitative methylation-specific real-time PCR (qMSP). The cycle threshold (Ct) values for targeted genes were normalized in each reaction using a primer/probe set for the reference gene, mC-LESS, a unique sequence that does not contain cytosines (27). Supplementary Table S2 lists primers and TaqMan probes (Applied Biosystems) for mC-LESS and the 3 genes examined. Each qMSP reaction batch was controlled with one positive (M. Sssl methylase–treated DNA) and multiple blanks with no DNA. For each plate, mC-LESS (the internal control) and the tested genes were determined together to avoid inter-assay variation. All qMSP reactions were conducted in triplicate and averaged.

Relative average telomere length measurement

Relative telomere length was measured as a comparative quantification, in particular as abundance of telomeric template versus single-copy gene (T/S) by quantitative real-time PCR as described previously (28). For the quantitative real-time PCR, the iTaq SYBR Green Supermix (Bio-Rad) and StepOnePlus Real-Time PCR System (Applied Biosystems) were used. The primers for telomeres and single-copy genes (H-globin) are listed in Supplementary Table S3. All measurements were carried out in duplicate and averaged.

Statistical analysis

Methylation levels and telomere length in whole blood DNA between gastric cancer and control subjects were compared using the Student t test. When the P values were less than 0.05, the logistic regression model was used to adjust for age. The correlation between methylation levels, telomere length, and age was assessed using the Spearman correlation analysis. Two-sided P < 0.05 was considered statistically significant.

Results

Study populations

Table 1 describes the age and gender of the gastric cancer, control, and healthy young individuals groups. In the comparison of gastric cancer and control subjects, the age of the gastric cancer subjects was significantly higher than that of the controls (control vs gastric cancer training set: P = 0.01; control vs gastric cancer validation set: P < 0.0001).
We did not find any association for the remaining 13 CpG
adjusted $P$ compared with the control group (11.3% vs 10.5%; age-
in the methylation of the control, and healthy young individuals groups examined
healthy young individuals (10.5
was not significantly different between controls and
mucosa (10), we determined whether $H. pylori$
fection status would influence the methylation status of whole blood DNA. $H. pylori$
status was available in 69 cancer-free subjects (4 young individuals and 65 controls)
and 71 patients with gastric cancer. As expected, $H. pylori$
infection was more prevalent in gastric cancer (61/70, 86%)
than in cancer-free subjects (29/43, 62.3%; $P = 0.0006$).
However, we did not find any significant association
between DNA methylation and $H. pylori$ infection status
(all $P$ values >0.1, data not shown).

**Methylation status of candidate genes in whole blood DNA in relation to aging**
We investigated the association between whole blood DNA methylation and aging using the Spearman correlation analysis. We combined the training set, control, and healthy young individuals groups. One hundred and ninety one subjects were included for this analysis. We found significant positive correlations between 8 CpG island promoters ($GDNF$, $CDH1$, $RARAB2$, $CDH13$, $MYOD1$, $SFRP1$, $SLC16A12$, $DPYS$, and $N33$) and $LINE1$ with aging. The methylation status of 2 genes ($DPYS$ and $N33$) showed a relatively strong correlation with higher age ($DPYS: R = 0.37, P < 0.0001; N33: R = 0.37, P < 0.0001$; Fig. 3A). Also, the Z score of mean methylation for the nine genes was closely correlated with higher age ($R = 0.41, P < 0.0001$; Fig. 3B).

**Telomere length in gastric cancer and control subjects and its relationship to aging and gene methylation**
We examined the relative telomere length of whole blood DNA with quantitative real-time PCR. Because of several gastric cancer subjects with short telomere length, the gastric cancer group showed shorter mean telomere length than the control group. However, this association was not significantly different (Fig. 4A). We investigated the clinicopathologic features of gastric cancer in 6 patients with gastric cancer with the lowest telomere length (histology, staging, location, etc.), but we did not find any significant associations. We also investigated the association of age and methylation status with telomere length. Shortened

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<th>Table 1. Study populations</th>
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<tr>
<td>Variables</td>
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<tr>
<td>$N$</td>
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<tr>
<td>Age (mean ± SEM)$^a$</td>
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<tr>
<td>Male$^b$</td>
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$^a$Control without gastric cancer versus gastric cancer training set, $P = 0.01$, Control without gastric cancer versus gastric cancer validation set, $P < 0.0001$.
$^b$Healthy young individuals versus gastric cancer validation set, $P = 0.006$.
telomere length was significantly correlated with higher age ($R = -0.26$, $P = 0.0003$; Fig. 4B). We also found significant inverse correlation between shortened telomere length and methylation of 4 CpG island promoters ($DPYS$, $CDH13$, $MYOD1$, and $SLC16A12$; Supplementary Fig. S2). A marginal significant correlation was also found between shortened telomere length and $Z$ score of the mean methylation of nine age-related sites ($GDNF$, $CDH1$, $RARAB2$, $CDH13$, $MYOD1$, $SFRP1$, $SLC16A12$, $DPYS$, $N33$, and $LINE1$; $R = -0.18$, $P = 0.01$; Fig. 4C).

**Methylation status of the $SFRP1$ gene in the gastric cancer validation set**

The $SFRP1$ promoter displayed a significant increase in methylation in the gastric cancer training set when compared with the control group. To confirm these data, we...
evaluated its methylation status in a gastric cancer validation set (n = 91) and found a significant though marginal increase of SFRP1 methylation in the gastric cancer validation set in comparison with the control group (11.5% vs 10.5%; age adjusted P value: P = 0.001; Fig. 5). We also investigated whether higher methylation of the SFRP1 gene...
is associated with clinicopathologic characteristics of gastric cancer using the combined training and validation sets (\( n = 158 \)); however, no significant association was found between \textit{SFRP1} methylation and any clinicopathologic features of gastric cancer (Table 2).

**Discussion**

In this study, we tested whether methylation analysis of selected loci in whole blood DNA could be useful as a biomarker of risk in gastric cancer. Among all the sites we tested, only a marginal increase in the methylation of the \textit{SFRP1} promoter was observed in both training and validation sets. The \textit{SFRP1} gene is located at 8p11.21 and its frequent methylation has been documented in colorectal cancer tissues as well as in aged non-neoplastic colon mucosa (25). Our results also suggest that the \textit{SFRP1} promoter is one of the regions where methylation in blood could reflect gastric cancer predisposition, but the differences between cases and controls are small and the data should be verified in other cohorts. Overall, our negative results stand in contrast to other studies. In recent studies on gastric cancer, the methylation levels of the \textit{IGF2} differentially methylated region 0 (DMR0) and the \textit{Alu} and \textit{LINE1} repetitive elements tended to be lower in blood from patients with gastric cancer than in control groups, but the association in overall subjects was not significant (\( P > 0.05 \) for all; refs. 29, 30). Another study explored the association between methylation in prediagnostic blood leukocyte DNA and gastric cancer risk in the prospective cohort (31). The result showed \textit{Alu} methylation was inversely associated with gastric cancer risk, mainly among cases diagnosed one or more years after. However, in overall subjects, methylation was not significantly different among the cases and controls (31). This issue has also been looked at in other tumors such as bladder cancer and others. Although several studies reported on promising results, the differences tended to be small and few of these have been validated by others. For example, white blood cells (WBC) DNA methylation of the \textit{LRE1} sequence and \textit{ATM} intragenic loci (ATMmvp2a) were significantly associated with the risk of head and neck and breast cancers (15, 17). However, the difference in methylation levels for cases and controls in both studies was not large (0.753 vs 0.747 for \textit{LRE1} in the head and neck cancers; 76.8% vs 76.4% and 76.9% vs 81.8% for 2 independent breast cancer cohorts; refs. 15, 17). There have also been several studies showing the possible role of constitutional methylation in blood DNA in cancer predisposition (14–17). Wong and colleagues reported that methylation of the \textit{BRCA1} promoter in blood DNA was more frequent in early-onset breast cancer patients and correlated with higher \textit{BRCA1} methylation in tumors and \textit{BRCA1} mutation associated with pathologic features (16). It seems unlikely that any of the markers analyzed here fit this constitutional methylation paradigm. Taken together, these results suggest that methylation in whole blood might reflect cancer predisposition. However, these changes are relatively small, with a large overlap between cases and
controls and the potential usefulness of blood DNA methylation as a screening/diagnostic biomarker for cancer may therefore be limited.

Our study had limitations worth discussing. The selection of genes was based on the hypothesis that the methylation status of targeted tissues (i.e., cancer and surrounding tissue) might be reflected in nontargeted tissues and correlate to cancer susceptibility. Therefore, we selected genes that have been reported to be hypermethylated in several cancers, including gastric cancer as well as in inflamed or aged tissues (9, 10, 20–26). It is possible that the methylation of other genes might serve as better markers of risk and this can be addressed in future studies using genome wide methylation analysis technologies (32, 33). Our data suggest that true differences might be small however, and these studies will need careful attention to sample size, validation, and quantitation to avoid the possibilities of false-positive findings. Another issue is technical, pyrosequencing is quantitative but has a background of approximately 5% methylated molecules, even those derived from circulating tumor DNA. All 3 genes showed higher methylation in gastric cancer tissues relative to healthy gastric mucosa (Supplementary Fig. S1). However, we did not find any difference in the methylation status of whole blood between gastric cancer and control subjects. It is also worth noting that whole blood DNA may include circulating DNA derived from tumor cells; it is likely to be in small amounts (<1% refs. 34, 35), but it may be a confounder in evaluating the significance of marginally positive results (such as for SFRP1). Finally, it is important to note that a formal study of a risk marker requires follow-up of individuals positive for that marker rather than a case–control study (as conducted here), and this is most relevant to positive results.

Our study confirms a firm association between aging and promoter CpG island methylation; 9 sites (GDNF, CDH1, RARAB2, CDH13, MYOD1, SFRP1, SLCA16A12, DPYS, N33, and LINE1) showed a significant increase in methylation throughout the aging process. The Z score of mean methylation for these 9 sites had a relatively good correlation with aging (R = 0.41, P < 0.0001). Moreover, methylation of the 9 sites was inversely associated with telomere length. Telomere length shortening has been observed in aged blood DNA and inflamed tissues and is considered to be associated with cell turnover (18, 19). Therefore, our results indicate that DNA methylation in the blood increases at many sites throughout the lifespan and this methylation increase is partly associated with the turnover of white blood cells. On the other hand, we did not find any difference in the mean methylation levels of these 9 age-related sites between gastric cancer and control groups (data not shown). Several other studies have examined age-related methylation in whole blood and found a number of sites that are hypermethylated with age (13, 36), whereas very few were associated with age-related phenotypes (31). This lack of association between the severity of age-related changes in DNA methylation and disease occurrence may be due to the tissue specificity of the link between aberrant methylation and disease, which may therefore not be detectable in whole blood. Alternatively, a link between methylation and disease is more likely if aberrant methylation is rate limiting. For cancer, it is possible that the rate-limiting step is acquisition of a mutation in a hypermethylated “field” (37, 38), a property that cannot be measured by DNA methylation alone. Although aging is the dominant factor in accounting for white blood cell methylation CpG island methylation, only a small fraction of the variation in DNA methylation in the blood remains unclear.

<table>
<thead>
<tr>
<th>Variables (n)</th>
<th>SFRP1 methylation (mean ± SEM%)</th>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male (110)</td>
<td>11.4 ± 0.26</td>
</tr>
<tr>
<td>Female (48)</td>
<td>11.6 ± 0.31</td>
</tr>
<tr>
<td>H. pylori status</td>
<td></td>
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<tr>
<td>Negative (38)</td>
<td>11.2 ± 0.46</td>
</tr>
<tr>
<td>Positive (120)</td>
<td>11.5 ± 0.23</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Intestinal (110)</td>
<td>11.5 ± 0.26</td>
</tr>
<tr>
<td>Diffuse (48)</td>
<td>11.3 ± 0.31</td>
</tr>
<tr>
<td>Location</td>
<td></td>
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<tr>
<td>Antrum (49)</td>
<td>11.9 ± 0.43</td>
</tr>
<tr>
<td>Body (81)</td>
<td>11.2 ± 0.25</td>
</tr>
<tr>
<td>Cardia (28)</td>
<td>11.4 ± 0.48</td>
</tr>
<tr>
<td>Staging</td>
<td></td>
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<tr>
<td>Early (82)</td>
<td>11.8 ± 0.33</td>
</tr>
<tr>
<td>Advanced (76)</td>
<td>11.2 ± 0.23</td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
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<tr>
<td>Elevated or protruding (40)</td>
<td>11.8 ± 0.44</td>
</tr>
<tr>
<td>Depressed or with ulceration (105)</td>
<td>11.3 ± 0.25</td>
</tr>
<tr>
<td>Scirrhous (13)</td>
<td>11.1 ± 0.55</td>
</tr>
</tbody>
</table>

NOTE: Methylation status was not determined for 5 cases.
Ehrlich M. DNA hypomethylation, cancer, the immunode

Maekita T, Nakazawa K, Mihara M, Nakajima T, Yanaoka K, Iguchi M,
Christensen BC, Houseman EA, Marsit CJ, Zheng S, Wrensch MR,
Takubo K, Izumiyama-Shimomura N, Honma N, Sawabe M, Arai T,
Issa JP. Aging, DNA methylation and cancer. Crit Rev Oncol Hematol
3.

Feinberg AP, Tycko B. The history of cancer epigenetics. Nat Rev


Eden A, Gaudet F, Waghmare A, Jaenisch R. Chromosomal instability


Ehrlich M. DNA methylation in cancer: too much, but also too little.

Ehrlich M. DNA hypomethylation, cancer, the immunodeficiency, cen-
tromic region instability, facial anomalies syndrome and chromoso-

Issa JP, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, Baylin SB.
Methylation of the oestrogen receptor CpG island links ageing and

Issa JP, Ahuja N, Toyota M, Bronner MP, Brentnall TA. Accelerated
age-related CpG island methylation in ulcerative colitis. Cancer Res

Maekita T, Nakazawa K, Mihara M, Nakajima T, Yanaoka K, Iguchi M,
et al. High levels of aberrant DNA methylation in Helicobacter pylori-
fected gastric mucosae and its possible association with gastric cancer

Christensen BC, Housman EA, Maris CJ, Zheng S, Wrensch MR,
Wiemels JL, et al. Aging and environmental exposures alter tissue-
specific DNA methylation dependent upon CpG island context. PLoS
Genet 2009;5:e1000602.

Teschendorff AE, Menon U, Gentry-Maharaj A, Ramsus SJ, Weisen-
berger DJ, Shen H, et al. Age-dependent DNA methylation of genes
that are suppressed in stem cells is a hallmark of cancer. Genome Res

Maris CJ, Koestler DC, Christensen BC, Karagas MR, Housman EA,
Kelsey KT. DNA methylation array analysis identifies profiles of blood-
derived DNA methylation associated with bladder cancer. J Clin

Hsing DT, Maris CJ, Housman EA, Eddy K, Furniss CS, McClean
MD, et al. Global DNA methylation level in whole blood as a biomarker
in head and neck squamous cell carcinoma. Cancer Epidemiol Bio-

Wong EM, Southey MC, Fox SB, Brown MA, Dowty JG, Jenkins MA,
et al. Constitutional methylation of the BRCA1 promoter is specifically
associated with BRCA1 mutation-associated pathology in early-onset

Brennan K, Garcia-Clasos M, Onn N, Fletcher O, Jones M, Ashworth A,

Takubo K, Izuimayama-Shimomura N, Honma N, Sawabe M, Arai T,
Kato M, et al. Telomere lengths are characteristic in each human

Genetic, epigenetic, and clinicopathologic features of gastric carcinomas with
the CpG island methylator phenotype and an association with Epstein-

Watanabe Y, Kim HS, Castoro RJ, Chung W, Estebro MR, Kondo K,
et al. Sensitive and specific detection of early gastric cancer with DNA

Age-related DNA methylation changes in normal human prostate

Yamamoto E, Toyota M, Suzuki H, Kondo Y, Sanomura T, Murayama
Y, et al. LINE-1 hypomethylation is associated with increased CpG
island methylation in Helicobacter pylori-related enlarged-fold gastri-

Identification of novel tumor markers in prostate, colon and breast

genetic and epigenetic analysis identifies three different subclasses of

Detection of bladder cancer using novel DNA methylation biomar-
kers in urine sediments. Cancer Epidemiol Biomarkers Prev 2011;

Weisenberger DJ, Trinh BN, Campan M, Sharma S, Long TI, Ana-
thrnarayan S, et al. DNA methylation analysis by digital bisulfite

McGrath M, Wong JY, Michaud D, Hunter DJ, De Vivo I. Telomere
length, cigarette smoking, and bladder cancer risk in men and women.

Insulin-like growth factor 2 hypomethylation of blood leukocyte
DNA is associated with gastric cancer risk. Int J Cancer 2012;
131:2596–603.

leukocyte DNA hypomethylation and gastric cancer risk in a high-risk

leukocyte Alu and LINE-1 methylation and gastric cancer risk in the

Estebro MR, Issa JP. Tackling the methylome: recent methodological
advances in genome-wide methylation profiling. Genome Med 2009;
1:106.

dNA methylation patterns in healthy blood cells and extensive
deletions in leukemia measured by a new quantitative technique.
Epigenetics 2012;7:1368–78.

Surgeon SR, Balasubramanian R, Schairer C, Muss HB, Ziegler RG,
Arcaro KF. Detection of promoter methylation of tumor suppressor
genes in serum DNA of breast cancer cases and benign breast disease

Prognostic role of methylated free circulating DNA in colorectal cancer.

Bell JT, Tsai PC, Yang TP, Pidsley R, Nisbet J, Glass D, et al. Epigen-
ome-wide scans identify differentially methylated regions for age and
age-related phenotypes in a healthy aging population. PLoS Genet 2012;
8:e1002629.

MGMT promoter methylation and field defect in sporadic colorectal

Niwa T, Ushijima T. Induction of epigenetic alterations by chronic
inflammation and its significance on carcinogenesis. Adv Genet 2010;

Association between folate levels and CpG Island hypermethyla-
tion in normal colorectal mucosa. Cancer Prev Res 2010;3:
1552–64.
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