Blood DNA Methylation As A Surrogate Epigenetic Biomarker In Study Of Night Shift Work And Breast Cancer

Yi Jin
jinyieking@gmail.com

Follow this and additional works at: https://elischolar.library.yale.edu/ysphtdl

Recommended Citation
https://elischolar.library.yale.edu/ysphtdl/1952

This Open Access Thesis is brought to you for free and open access by the School of Public Health at EliScholar – A Digital Platform for Scholarly Publishing at Yale. It has been accepted for inclusion in Public Health Theses by an authorized administrator of EliScholar – A Digital Platform for Scholarly Publishing at Yale. For more information, please contact elischolar@yale.edu.
Blood DNA methylation as a surrogate epigenetic biomarker in study of night shift work and breast cancer

Name: Yi Jin

Year Completed: 2020

Year Degree Awarded: 2020

Degree Awarded: Master of Public Health

Department: School of Public Health

Advisor/Committee Chair: Yong Zhu

Committee Members: Zuoheng Anita Wang
Table of Contents

Abstract 3
List of Tables 4
List of Figures 5
Introduction 6
Materials and methods 8
  Study population 8
  Data collection 9
  Statistical analysis 10
Results 12
Discussion 21
References 23
Abstract

**Objectives:** The objective was to explore the consistency of DNA methylation pattern between peripheral blood and breast tumor tissue, and discussed the potential of using blood as a surrogate epigenetic biomarker to study the relationship between breast cancer and shiftwork.

**Methods:** Systematic review on studies analyzing the concordance or correlation of DNA methylation level between breast tumor tissue and peripheral blood was conducted. The results of eligible studies were summarized to find the specific genes that had consistent methylation pattern in both tumor tissues and blood. Then the blood samples from the well-established Danish cohort were used to perform epigenome-wide association analysis to show the genome-wide methylation pattern in the blood. Cohort studies on each gene and its relationship between breast cancer were searched to discuss whether that gene might be a precursor for breast cancer.

**Results:** The DNA methylation array of the Danish cohort in this study detected 5409 CpG sites that differentially methylated between day workers and night shift workers. Totally 4750 genes showed significantly differential methylation level between the two groups. Among 22 candidate genes and 3 repetitive elements from the eligible studies and public datasets, methylation patterns of the five genes, IGF2, BRCA1, GSTP1, P16 and MGMT were consistent between blood and breast tumor tissues among breast cancer patients and showed significant difference between day shift workers and night shift workers, which implicated that it could be potential biomarkers for the screening of breast cancer and as a surrogate to study the relationship between breast cancer and circadian disruption.

**Conclusion:** Aberrant methylation status of five genes, IGF2, BRCA1, GSTP1, P16 and MGMT, might be a promising epigenetic biomarker for early detection and diagnosis of breast cancer, and it can also be a surrogate biomarker to study the relationship between breast cancer and night shiftwork.

**Keywords:** DNA methylation, breast cancer, night shiftwork, Chronic Disease Epidemiology
List of Tables

TABLE 1. DESCRIPTIONS OF THE INCLUDED STUDIES 12
TABLE 2. SUMMARY OF THE CANDIDATE GENES OVERLAPPED IN THE TWO GENE SETS 14
TABLE 3. CHARACTERISTICS OF STUDY POPULATION IN DANISH COHORT 15
List of Figures

FIGURE 1. VENN DIAGRAM: OVERLAPPED GENES IN THE TWO DATASETS

16
Introduction

Breast cancer (BC) is the most common cancer in women around the world, and accounts for 15% of cancer deaths among women, which is the leading cause of cancer death in females worldwide. (1) Early detection and diagnosis of breast cancer has ignited broad discussion among scientists during the past several decades. Some studies showed that the epigenetic alterations, especially DNA methylation of repetitive elements or specific genes, might be potential biomarkers for breast cancer detection. Night shift work has long been assumed to be a risk factor for breast cancer. Some studies showed an elevated incidence of breast cancer among night shift workers. (2) How night shift work impacts the carcinogenesis of breast cancer remains unclear. Here we hypothesized that night shift work might be associated with DNA methylation of certain genes that are related to breast cancer.

DNA methylation is an important epigenetic mechanism involving direct chemical modification to DNA that can affect genetic performance without changing DNA sequences. It refers to the process of a methyl group covalently bonded to the 5' carbon position of the cytosine under the action of DNA methyltransferases (DNMTs) to form 5-methylcytosine (5mC). DNA methylation typically occurs at CpG islands that possess functional importance. (3) Numerous studies have shown that DNA methylation can cause changes in chromatin structure, DNA conformation, stable gene silencing, and the way DNA interacting with proteins, thereby controlling gene expressions. Aberrant DNA methylations, including hypermethylation within promotor regions that followed by inactivation of tumor-suppressor genes and global hypomethylation inducing genomic instability or overexpression of oncogenes, are involved in carcinogenesis of different cancer types, including breast cancer. (4) DNA methylation also has high value in detecting and preventing breast cancer.
The development of adjuvant treatment combined with promotion of screening programs and advanced diagnostic techniques have improved the outcome of breast cancer and decreased the mortality rate of breast cancer during the past couple of decades. However, there are still around one third of breast cancer patients developing metastasis and eventually died from the disease. (5) Therefore, more effective methods to detect breast cancer at an early stage are needed.

Most recently, there have been many studies on using blood as a surrogate epigenetic biomarker for the detection and diagnosis of breast cancer. Several studies showed correlation of methylation patterns between peripheral blood samples and breast tumor tissues. If further study can confirm the concordance between the two tissues, blood may be utilized as a promising biomarker for screening and diagnosis. It is obvious that blood as a surrogate biomarker has several distinct advantages. It is non-invasive, and comparing to biopsy blood test is more convenient and accessible. Most existing studies tested the association between DNA methylation patterns in the blood and breast cancer risk, and did not focus on the consistency between blood and breast tissues. Therefore, the evidence that DNA methylation level in the blood was associated with breast cancer was not sufficient to make the inference that DNA methylation in the peripheral blood can represent DNA methylation pattern in the breast tumor tissues, thus can be used as a surrogate biomarker to detect breast cancer for individuals.

Therefore, this study has two specific objectives. The first objective of this study is to explore the consistency of DNA methylation pattern between blood and breast tumor tissues, and detect the candidate genes of which the DNA methylation level could be used to predict breast cancer. The second objective is to observe whether night shift work is significantly associated with aberrant methylation of those candidate genes using genome-wide methylation analysis.
Materials and methods

Study population

*Population in the selected studies*

In the systematic review, participants in the selected studies were female breast cancer patients and normal controls. Some of the studies were case-only, and no normal controls were included, of which the comparison of DNA methylation level was observed among breast tumor tissue, peripheral blood and normal breast tissue. The details of the studies were listed in Table 1.

*Population in the Danish cohort*

The blood samples for the DNA methylation assay in this study were collected from the participants of the Danish prospective cohort study “Diet, Cancer and Health”. In the Danish cohort study, all the female subjects were recruited between December 1993 and May 1997, and followed forward. The subjects recruited were aged 50 to 64 years old, with no diagnosis of any cancer registered in the Danish Cancer Registry, and born in Denmark. The information on lifestyles and other characteristics including shift work were gathered through questionnaire and interview. Participants provided their blood sample after the end of visit to the study center. Females participants that met the following criteria were selected: 1) had blood samples that were available for methylation analysis; 2) provided information on history of shift work. Shift work was defined as working starting from 19:00h or later and ending before 09:00h. Totally 117 female participants were eligible for this study. Among the 117 eligible participants, 19 of them are exposed to long-term (defined as ≥ 10 years of history) night shift work and the remaining 98 were day workers who had never been exposed to shift work. Age and total folate intake were not significantly differed between night shift workers and day workers. In our study, we randomly
selected 10 night shift workers from 19 long-term shift workers and 10 day workers were selected matching on age (± 2 yrs) and total folate intake (± 55 μg/day). All the blood samples collected were frozen and stored at -150 Celsius. Details of the Danish cohort study and the procedures of collecting blood sample were described in the previous study. (6)

Data collection

Data from the systematic review on concordance between breast tissue and peripheral blood

Papers were read through to decide if the study is eligible for further review. After being selected, the following information of the studies was listed: study, sample type, sample size, and candidate genes or array. Each gene that met the following criteria were analyzed in our Danish cohort: 1) showed significant different DNA methylation level between breast tumor tissue and normal breast tissue if comparison with normal breast tissues were applicable in the study, and 2) had a significant correlation or concordance of DNA methylation pattern between breast tumor tissue and peripheral blood, including leukocytes, plasma and serum.

DNA methylation procedure and assays of the Danish cohort

DNA methylation array of the blood samples from 10 day-shift workers and 10 night-shift workers were generated with Illumina 27k. The details of the method were previously described in a pilot study Zhu11 (7). In the study, Illumina Infinium Methylation Assay was used for genome-wide methylation array, with 50 ng of genomic DNA gathered from each subject, and totally 27,578 CpG sites spanning 14,495 genes were covered. CpG sites that tested in this study were located with the proximal promoter regions, with average distance 389 bp from the transcription start site.
Review on cohort studies of association between each overlapped gene and breast cancer

Cohort studies of each genes overlapping in both gene sets will be searched. Review on the cohort studies will be performed to discuss whether that gene might be a precursor of breast cancer. This review is to provide some insight to the relationship between DNA methylation and breast cancer for further study.

Statistical analysis

Concordance based on the previous studies

The concordance or correlation of DNA methylation between breast tumor tissues and blood (including white blood cells, leukocytes, plasma and serum) was summarized using the correlation coefficient in the original study. Studies using methylation frequency as a measurement or other measurement that did not provide parameters on correlation were excluded from further analysis.

DNA methylation levels of the candidate genes from the Danish cohort associated with night shift work

DNA methylation level of the selected samples from the Danish cohort was output through software Illumina. The difference of DNA methylation level between day workers and night-shift workers was analyzed using student t-test in GenomeStudio of Illumina, Inc. All the genes selected in the systematic review were analyzed independently. Each selected gene was searched in the methylation dataset of Danish cohort, and its difference of methylation level between day workers and night shift workers was checked for significance. Pathway analysis on the interactions of the methylated genes were performed
to further study the impact of night shift work on biological pathway using software tool Ingenuity Pathway Analysis (IPA).

**Overlapping of genes from the two studies**

If the association between breast cancer and DNA methylation of the candidate genes that had significant concordance of DNA methylation between breast tumor tissue and blood can be verified by our statistical analysis of the DNA methylation pattern in Danish cohort, Venn’s diagram was utilized to visualize the overlap between the two groups of genes. The genes that appears in both groups might be used as potential epigenetic biomarkers to detect breast cancer, and associated with night shift work.
**Results**

*Systematic review of concordance of DNA methylation patterns between breast tumor tissue and blood samples for breast cancer patients*

Table 1. Descriptions of the included studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Geography</th>
<th>Tissue type</th>
<th>Sample size</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wojdacz et al. (8)</td>
<td>2011</td>
<td>Denmark</td>
<td>Breast tumor tissue</td>
<td>75</td>
<td>Gene-specific methylation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peripheral blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barault et al (9)</td>
<td>2013</td>
<td>America</td>
<td>Breast tumor tissue</td>
<td>35</td>
<td>Gene-specific methylation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peripheral blood</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Cho (10)</td>
<td>2010</td>
<td>Turkey</td>
<td>Breast tumor tissue</td>
<td>40</td>
<td>Gene-specific methylation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peripheral blood</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Cho (11)</td>
<td>2015</td>
<td>America</td>
<td>Breast tumor tissue</td>
<td>952</td>
<td>Gene-specific methylation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peripheral blood</td>
<td>1021</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1036</td>
<td></td>
</tr>
<tr>
<td>Radpour et al. (12)</td>
<td>2011</td>
<td>Switzerland</td>
<td>Breast tumor tissue</td>
<td>20</td>
<td>Gene-specific methylation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Blood (serum)</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal breast tissue</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Prajjendanz et al. (13)</td>
<td>2020</td>
<td>Poland</td>
<td>Breast tumor tissue</td>
<td>262</td>
<td>Gene-specific methylation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peripheral blood</td>
<td>942</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>500</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2010</td>
<td>India</td>
<td>Breast tumor tissue</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
Totally 524 articles were found in PubMed with keywords “DNA methylation; blood; breast cancer” from 1991 to March 11th, 2020. Related articles were also reviewed for eligibility. After reviewing the title and abstract of articles, 42 papers were selected. 7 of the 42 studies met our criteria for further study after reading the whole paper. In these 7 papers, only four studies indicated significant concordance or correlation of methylation level of specific genes between blood and breast tumor tissue. In these seven studies, Woj11 and Pra20 used methylation-sensitive high-resolution melting (MS-HRM) and PCR to measure the DNA methylation status, Bar13 used PCR and highly quantitative bisulfite pyrosequencing, Cho10 and Cho15 used MethyLight and PCR, Rad11 used PCR and mass spectrometry to measure the methylation proportion, and Sha10 used methylation-sensitive PCR (MS-PCR) to measure the methylation status.

In the eligible studies, totally 22 candidate genes and 3 repetitive elements were studied and their methylation levels were measured and compared among different tissues. Among the 22 candidate genes and differentially methylated regions (including BRCA1, APC, RASSF1A, GRB10, H19, KvDMR, SNRPN/SNURF, IGF2, HIN1, CDH1, RARβ, TWIST1, CyclinD2 [CCND2], BIN1, BMP6, CST6, ESR-b, GSTP1, P16 [CDKN2A], P21 [CDKN1A], TIMP3, MGMT), 8 genes, IGF2 (including IGF2 DMR0 and IGF2 DMR1), BMP6, BRCA1, CST6, GSTP1, P16 (alias: CDKN2A), TIMP3 and MGMT were significantly correlated between blood and breast tumor tissue in breast cancer patients. Two of the three repetitive elements in the selected studies, LINE1 and Sat2M1, also showed significant concordance between blood and breast tumor tissue. Since our array did not measure repetitive elements, these two repetitive elements were not eligible for further analysis.
<table>
<thead>
<tr>
<th>Gene / Repetitive elements</th>
<th>Study</th>
<th>Statistical methods</th>
<th>Systematic review</th>
<th>Day-shift comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Correlation</td>
<td>p-value</td>
<td>Shift.DiffScore (largest absolute value)</td>
</tr>
<tr>
<td>IGF2 DMR0 Bar13</td>
<td>Spearman’s correlation</td>
<td>0.65</td>
<td>0.0006</td>
<td>78.14096</td>
</tr>
<tr>
<td>IGF2 DMR2 Bar13</td>
<td>Spearman’s correlation</td>
<td>0.50</td>
<td>0.0261</td>
<td></td>
</tr>
<tr>
<td>BMP6 Rad11</td>
<td>R-square linear</td>
<td>7.5E-4</td>
<td>&lt;0.05</td>
<td>4.929239</td>
</tr>
<tr>
<td>BRCA1 Rad11</td>
<td>R-square linear</td>
<td>0.307</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>BRCA1 Pra20</td>
<td>-</td>
<td>-</td>
<td>&lt;0.001</td>
<td>25.60556</td>
</tr>
<tr>
<td>BRCA1 Sha10</td>
<td>r</td>
<td>0.94</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>CST6 Rad11</td>
<td>R-square linear</td>
<td>0.078</td>
<td>&gt;0.05</td>
<td>0.964586</td>
</tr>
<tr>
<td>GSTP1 Rad11</td>
<td>R-square linear</td>
<td>0.365</td>
<td>&lt;0.05</td>
<td>-33.06441</td>
</tr>
<tr>
<td>GSTP1 Sha10</td>
<td>r</td>
<td>0.90</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>P16 (CDKN2A) Rad11</td>
<td>R-square linear</td>
<td>0.014</td>
<td>&lt;0.05</td>
<td>23.98066</td>
</tr>
<tr>
<td>TIMP3 Rad11</td>
<td>R-square linear</td>
<td>0.18</td>
<td>&lt;0.05</td>
<td>-5.571209</td>
</tr>
<tr>
<td>MGMT Sha10</td>
<td>r</td>
<td>0.80</td>
<td>&lt;0.001</td>
<td>-64.26</td>
</tr>
<tr>
<td>LINE1 Cho10</td>
<td>Spearman’s correlation</td>
<td>0.46</td>
<td>0.003</td>
<td>-</td>
</tr>
</tbody>
</table>
**DNA methylation pattern in the peripheral blood of Danish cohort**

Characteristics of the study population selected from Danish cohort was described in the Table 3. Age and total folate intake of the day workers and night shift workers were not significantly different.

**Table 3. Characteristics of study population in Danish cohort**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Day workers (98, 83.8%)</th>
<th>Night shiftworkers (19, 16.2%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>55.17±0.33</td>
<td>55.26±0.91</td>
<td>0.9168</td>
</tr>
<tr>
<td>(mean ± SEM years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total folate intake</td>
<td>373.2±14.57</td>
<td>367.4±34.75</td>
<td>0.8720</td>
</tr>
<tr>
<td>(mean ± SEM μg/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the analysis of DNA methylation array, totally 5409 CpG sites spanning over 4750 genes were found to have differential methylation level between day workers and long-term night shift workers. Among all the significantly differentially methylated CpG sites, 66.4% of them corresponding to 3593 methylated loci spanning 3196 genes, were hypermethylated, which means that the methylation level was higher than the normal, while the remaining 33.6% (1816 loci spanning 1613 genes) were hypomethylated, which means lower level than the normal.

**Overlapping genes of the two gene sets**
The overlapped genes between the DNA methylation array and eligible genes in the systematic review were found by manually searching for the genes in our dataset. Totally seven of the eight genes can be found in our gene sets without any cut-off. Significant level of the different methylation level was set at $|\text{DiffScore}| > 13$. Five genes, BRCA1, IGF2, GSTP1, P16 (CDKN2A) and MGMT, appeared in both gene sets and showed significant different methylation level between day workers and night shift workers.

Figure 1. Venn diagram: overlapped genes in the two datasets

The yellow circle represents the genes from our DNA methylation array that had different methylation level between day and night shift workers. 4750 genes were significantly differentially methylated between the 10 pairs of day workers and night shift workers. The blue circle represents the genes included in the systematic review. The red circle represents the genes of which the methylation level was significantly correlated between peripheral blood and breast
tumor tissues in breast cancer patients. The overlapped deep color circle represents the genes that appears in both datasets. The areas of the circles did not reflect the proportion of genes in different gene sets.

The pathway analysis in Zhu11 (7) showed that the network of interactions, designated as “DNA replication, recombination, and repair, gene expression, behavior” by IPA software, was significantly enriched in 35 genes that were differentially methylated in night shift workers compared to day workers. Interestingly, two genes appearing in the overlapped sets, P16 (CDKN2A) and MGMT were also among the aforementioned 35 genes.

**Review on cohort studies that explored the relationship between methylation of specific genes and breast cancer**

Keywords were each gene symbol plus “methylation; breast cancer; cohort”. Studies were from 1998 to March 11th, 2020.

**IGF2.** Keywords “IGF2; methylation; breast cancer” was used instead because of the limit amount of available papers in PubMed. Ito08 used the blood sample collected 2-5 years prior to breast cancer diagnosis from EPIC cohort and screened them to measure the hypomethylation status of IGF2. (15) The results found that IGF2 hypomethylation was not constitutively present in the blood before the diagnosis of breast cancer. It suggested that hypomethylation of IGF2 might be an acquired epimutation rather than precursor for breast cancer.

**BRCA1.** Totally 58 studies were found using the keywords “BRCA1; methylation; breast cancer; cohort”. There were inconsistent results among the few studies that provided information on association between BRCA1 promoter methylation and breast cancer development. A study in Vietnamese women
with breast cancer (16) gathered 79 pairs of breast tumor samples and matched adjacent normal breast tissues from women who underwent mastectomy at the Department of Pathology, National Cancer Hospital K, Hanoi, between 2014 and 2015. The investigators tested the significance of differential methylation frequency of BRCA1, RASSF1A and GSTP1 between breast tumor tissues and adjacent normal tissue, and found significantly higher methylation frequency of GSTP1 in breast tumor tissue than that in matched normal tissues, but no significantly differential methylation level of BRCA1. In Pra20 (13), which was also included in our systematic review, BRCA1 methylation status was significantly associated with breast cancer among triple-negative breast cancer patients as well as in ER-negative breast cancer patients, but among the overall patients, there was no significant association between BRCA1 methylation and breast cancer risk. It implicated that BRCA1 methylation might be a biomarker for clinicopathological characteristic rather than diagnosis or detection for breast cancer. In a meta-analysis (17) of four prospective cohort studies including MCCS, EPIC-Italy, EPIC-IARC and PLCO, BRCA1 promoter methylation in blood was not significantly associated with breast cancer risk (p=0.88).

**GSTP1.** Aside from the VuT18 (16) aforementioned, a case-control study on a cohort of Egyptian females (18) showed that GSTP1 methylation status was significantly higher in breast cancer patients than that in normal controls. One case-control study (19) nested in prospective New York University Women’s Health Study cohort found no significantly differential methylation of GSTP1 in serum between cases and controls.

**P16 and MGMT.** No eligible studies were found aside from the studies include in our systematic review.

*Summary of the functions of those genes in the carcinogenesis of breast cancer*
IGF2 gene participates in the expression of a protein called insulin-like growth factor 2 (IGF2) that plays important role in growth and development during embryogenesis and promoting the cell growth and cell proliferation in different tissues including breast tissues. IGF2 is also an imprinted gene, of which only the paternally inherited copy is active in cells. (20) IGF ligands (including IGF1 and IGF2) contributed to tumorigenesis of various types of cancer by binding to and activating IGF-1R, then lead to two signaling pathways: PI3K/AKT and Ras/MAPK. In estrogen receptor positive (ER+) breast cancer and HER2 positive breast cancer, studies showed that IGF signaling might be associated with drug resistance. (21)

BRCA1 germline mutation plays critical role in the development of breast cancer and ovarian cancer and its function was well-studied in previous studies. Although there are inconsistent results on the association between BRCA1 methylation and breast cancer risk, BRCA1 is one of the most studies genes in carcinogenesis, especially its role in breast cancer and ovarian cancer. BRCA1 and BRCA2, the breast cancer susceptibility genes, are tumor suppressor genes, and their mutation can lead to malignancies including various types of cancers. BRCA1 has been proved by the large amount of studies as the genes that participated in regulating DNA repair and preventing uncontrolled cell growth that might lead to cancer. Studies showed that BRCA1 participated in the tumorigenesis though suppressing IGF-1R promoter activity and decreasing endogenous IGF-1R level in breast-cancer cell line. (22) There was experimental evidence on the relations between BRCA1 mutations and dysregulation of the progesterone/RANK/RANKL/OPG system that might play an important role in carcinogenesis of breast cancer. (23)

GSTP1 encoded protein belongs to GSTs, a family of enzymes that participates in detoxification. GSTP1 proteins are thought to play important role in xenobiotic metabolism and susceptibility to some diseases.
including cancer. One study showed that GSTP1 was associated with activation of AMPK thereby impairing the oncogenic signaling pathway, and it was also involved in the inhibition of mTOR signaling. (24)

P16, also called CDKN2A, is a gene that is frequently mutated or deleted in a wide range of tumors, and is thought to be a tumor suppressor gene. In a study on ER-positive, HER2-negative breast cancer, investigators found that loss of CDKN2A (p16) was correlated with abnormal CDK4/6/RB pathway, which could lead to deregulated kinase activity and development of diseases including cancer. (25)

MGMT is known to be a DNA repair gene. The protein encoded by MGMT catalyzes the reaction of transferring methyl groups from O(6)-alkylguanine and other methylated moieties of the DNA to its own molecule thereby repairs the toxic lesions. Methylation of MGMT promoter is associated with diseases such as colorectal cancer, lung cancer and breast cancer. MGMT was involved in DNA damage signaling pathway by reversing alkylation. (26)
Discussion

Methylation status of five genes, IGF2, BRCA1, P16 (CDKN2A), GSTP1 and MGMT showed significant concordance between peripheral blood and breast tumor tissues in breast cancer patients. This result implicated that these genes might be utilized as potential biomarkers for non-invasive detection and diagnosis of breast cancer. However, some limitations of this study need to be specified. First, some genes displayed inconsistent differential methylation pattern in different studies. For example, BCRA1 methylation was significantly correlated between blood and breast cancer in Pra20 (13) among triple-negative breast cancer patients and ER-negative breast cancer patients, but for all the samples in the study regardless of their clinicopathologic status the association of BRCA1 methylation in blood sample with breast cancer was not significant. In our systematic review, subgroup analysis on clinical stage and subtypes of cancer was not conducted because information was limited or sample size was too small. In Rad11, another cohort consisting 36 plasma samples from breast cancer patients and 30 plasma samples from healthy women showed that methylation proportion of BRCA1, CST6, GSTP1, P16 and TIMP3 were significantly higher in breast cancer patients than that in normal controls. However, in Bro10, a nested case-control study aforementioned, found no significant differential GSTP1 methylation pattern between cases and controls. Second, blood samples and tumor tissues in some the studies, such as Pra20 and Cho15, was not collected from the same subject, which might introduce bias due to individual difference. But these two studies had sufficient sample size to detect population level association. Besides, the different types of blood sample might also affect the results. Rad11 used plasma and serum, Sha10 used serum, while Bar13 measured leukocyte DNA in peripheral blood. Different methods used to measure the methylation pattern were also possible to introduce bias because technologies had different coverage of CpG sites and sensitivity as well as parameter. However, due to the limited number of studies, subgroup analysis on different types of blood samples and methods was not feasible.
Based on our dataset, whether there is causal relationship between night shift work and DNA methylation was not clear and no temporality of the two events was established, thereby further studies using prospective cohort study design might be able to answer this question. According to the pilot study (7) using the same dataset with this study did show significant association between night shift work and DNA methylation of some genes.

In this study, review on prospective studies that study DNA methylation and breast cancer was also included. For each gene, there is limited amount of studies available in the PubMed. From the eligible studies, either there was no information on temporality of methylation status and breast cancer onset, or the evidence showed no significant association between methylation and breast cancer risk. Therefore, we cannot make any conclusion on the potential precursor of the genes discussed abovementioned. To answer the unsolved questions in this study, prospective cohort study with large sample size needs to be carried on.
References