Environmental Research 145 (2016) 93–100

Contents lists available at ScienceDirect

Environmental Research

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

Sources of polycyclic aromatic hydrocarbons are associated with gene-specific promoter methylation in women with breast cancer

Alexandra J. White [*,a], Jia Chen [b,c,d], Susan L. Teitelbaum [b], Lauren E. McCullough [a], Xinran Xu [b,e], Yoon Hee Cho [f], Kathleen Conway [a], Jan Beyea [g], Steven D. Stellman [i], Susan E. Steck [l], Irina Mordukhovich [a], Sybil M. Eng [i], Mary Beth Terry [i], Lawrence S. Engel [a], Maureen Hatch [m], Alfred I. Neugut [i,j], Hanina Hibshoosh [k], Regina M. Santella [h], Marilie D. Gammon [a]

[a] Department of Epidemiology University of North Carolina, Chapel Hill, NC, USA
[b] Departments of Preventive Medicine, New York, NY, USA
[c] Departments of Oncological Science, New York, NY, USA
[d] Departments of Pediatrics, Ichan School of Medicine at Mt. Sinai, New York, NY, USA
[e] Departments of Biometrics, Roche Product Development in Asia-Pacific, Shanghai, China
[f] Department of Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, MT, USA
[g] Department of Consulting in the Public Interest (CIPI), Lambertville, NJ, USA
[h] Departments of Environmental Health Sciences; Columbia University, New York, NY, USA
[i] Departments of Epidemiology, Columbia University, New York, NY, USA
[j] Departments of Medicine, Columbia University, New York, NY, USA
[k] Departments of Pathology and Cell Biology, Columbia University, New York, NY, USA
[l] Department of Epidemiology and Biostatistics, University of South Carolina, Columbia, SC, USA
[m] Departments of Division of Cancer Epidemiology and Genetics, Radiation Epidemiology Branch, National Cancer Institute, Bethesda, MD, USA

ARTICLE INFO

Article history:
Received 12 October 2015
Received in revised form
24 November 2015
Accepted 27 November 2015

Keywords:
Methylation
Smoking
Environmental tobacco smoke
Air pollution
Grilled meat.

ABSTRACT

Background: Tobacco smoke, diet and indoor/outdoor air pollution, all major sources of polycyclic aromatic hydrocarbons (PAHs), have been associated with breast cancer. Aberrant methylation may be an early event in carcinogenesis, but whether PAHs influence the epigenome is unclear, particularly in breast tissue where methylation may be most relevant. We aimed to evaluate the role of methylation in the association between PAHs and breast cancer.

Methods: In a population-based case-control study, we measured promoter methylation of 13 breast cancer-related genes in breast tumor tissue ($n=765$–851 cases) and global methylation in peripheral blood (1055 cases/1101 controls). PAH sources (current active smoking, residential environmental tobacco smoke (ETS), vehicular traffic, synthetic log burning, and grilled/smoked meat intake) were evaluated separately. Logistic regression was used to estimate adjusted odds ratios (ORs) and 95% confidence intervals (CIs).

Results: When comparing methylated versus unmethylated genes, synthetic log use was associated with increased ORs for *CDH1* (OR=2.26, 95%CI=1.06–4.79), *HIN1* (OR=2.14, 95%CI=1.34–3.42) and *RARβ* (OR=1.80, 95%CI=1.16–2.78) and decreased ORs for *BRCA1* (OR=0.44, 95%CI=0.30–0.66). Residential ETS was associated with decreased ORs for *ESR1* (OR=0.74, 95%CI=0.56–0.99) and *CCND2* methylation (OR=0.65, 95%CI=0.44–0.96). Current smoking and vehicular traffic were associated with decreased ORs for *DAPK* (OR=0.53, 95%CI=0.28–0.99) and increased ORs for *TWIST1* methylation (OR=2.79, 95% CI=1.24–6.30), respectively. In controls, synthetic log use was inversely associated with LINE-1 (OR=0.59, 95%CI=0.41–0.86).

Discussion: PAH sources were associated with hypo- and hypermethylation at multiple promoter regions in breast tumors and LINE-1 hypomethylation in blood of controls. Methylation may be a potential biologic mechanism for the associations between PAHs and breast cancer incidence.

Published by Elsevier Inc.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are established carcinogens to the lung (IARC, 2010), but their relationship with

* Corresponding author.
E-mail address: whitea@unc.edu (A.J. White).

http://dx.doi.org/10.1016/j.envres.2015.11.033
0013-9351/Published by Elsevier Inc.

breast cancer is not as well studied. PAH biomarkers, which tend to reflect recent exposure, have been associated with breast cancer incidence (Gammon et al., 2004b). Additionally, PAHs induce mammary tumors in laboratory animals (Hecht, 2002). PAHs are ubiquitous pollutants that form as a combustion by-product of organic material (Boström et al., 2002). The major sources of PAH in the general population are indoor and outdoor air pollution, tobacco smoke and diet (Boström et al., 2002). Elevated associations with breast cancer incidence have been observed with the main sources of PAH exposure, including active cigarette smoking (Gaudet et al., 2013), long-term environmental tobacco smoke (ETS) (Gammon et al., 2004a; Morabia et al., 1996), indoor air pollution from burning synthetic logs (White et al., 2014), outdoor air pollution (Hystad et al., 2015; Mordukhovich et al., 2015; Nie et al., 2007), and intake of grilled and smoked foods (Fu et al., 2011; Steck et al., 2007).

Aberrant DNA methylation, an epigenetic modification, plays an important role in breast carcinogenesis (Xu et al., 2011; Xu et al., 2012). Higher levels of methylation at promoter regions can silence tumor suppressor genes and tumor tissue may be were methylation is most relevant (Jones, 2012). Our research team has reported that methylation at promoter regions of specific genes in breast tumor tissue is associated with breast cancer clinical/pathological factors and mortality in a population-based sample (Cho et al., 2012). In contrast, lower levels of global methylation may confer genomic instability and increased mutation rates (Brennan and Flanagan, 2012). Although the associations between cancer and global methylation have been inconclusive (Brennan and Flanagan, 2012), our research group has previously reported an association with breast cancer for luminometric methylation assay (LUMA), but not for methylation of long interspersed elements-1 (LINE-1) in white blood cells (Xu et al., 2012). DNA methylation may be altered in response to environment and lifestyle factors and may be a potential biologic mechanism for disease (Bollati and Baccarelli, 2010). Other investigators have found that exposure to the PAH sources of tobacco smoke and air pollution may be associated with changes in DNA methylation patterns (Duan et al., 2013; Shenker et al., 2013).

For the current study, we aimed to better understand the role methylation plays in the PAH and breast cancer association. We first aimed to examine whether five individual PAH sources, previously found to be modestly associated with breast cancer incidence (current active cigarette smoking (Gammon et al., 2004a), long-term residential ETS (Gammon et al., 2004a), total grilled/smoked food intake (Steck et al., 2007), residential burning of synthetic logs (White et al., 2014) and high vehicular traffic exposure (Mordukhovich et al., 2015)), were also associated with promoter methylation status in a panel of 13-breast cancer related genes measured in the tumor tissue of a population-based sample of women with breast cancer. We also aimed to investigate whether these same PAH sources were associated with global methylation in a population-based sample of women without breast cancer, using two independent global methylation markers, LINE-1 and LUMA, measured in peripheral blood DNA.

2. Materials and methods

Our study builds upon population-based resources from the Long Island Breast Cancer Study Project (LIBCSP). The parent LIBCSP methods have been previously published in detail (Gammon et al., 2002). Institutional Review Board approval was obtained from all relevant institutions.

2.1. Study population

Study participants included 1508 breast cancer cases and 1,556 controls who were English-speaking women residing in Nassau and Suffolk counties on Long Island, New York. Written informed consent was obtained from all study participants.

Cases were women who had been recently diagnosed with a first primary *in situ* or invasive breast cancer between August 1st, 1996 and July 31st, 1997, and were residents of Nassau or Suffolk counties on Long Island, New York (NY). There were no age or race restrictions for case eligibility. Cases were identified using rapid case ascertainment from the pathology departments of all 28 hospitals on Long Island and three tertiary care hospitals in New York City. Diagnoses were confirmed by the physician or the medical record.

Controls had no prior history of breast cancer and were frequency matched in 5-year age groups to cases based on the expected age distribution of case women. Controls were identified in 1996–1997 from among adult female residents of Nassau and Suffolk counties in NY using random digit dialing for those who were less than 65 years of age, and for those who were 65 years of age and greater, using the Health Care Finance Administration rosters.

Study participants ranged in age from 20–98 years, and most cases and controls were postmenopausal (68.1% and 66.3%, respectively) and identified themselves as white (93.8% and 91.8%, respectively); the racial distribution of our population-based sample reflects that of Nassau and Suffolk counties at the time of data collection (Gammon et al., 2002). Cases and controls had similar distributions of education and income (Gammon et al., 2002), and the median age at menarche was also similar (12.6 years, standard deviation (SD)=1.67; and 12.6 years, SD=1.65, respectively). On average, controls were more likely to be parous than cases (89.0% versus 86.9%, respectively) (Gammon et al. 2002). Some 10% of cases and 8% of controls reported drinking 1–2 glasses per day (15–30 g) of alcohol, on average across the life course (Terry et al. 2006).

2.2. PAH exposure sources assessment

Five PAH exposure sources were assessed. Current active smoking, residential ETS, grilled/smoked meat intake, and synthetic log burning were assessed by a trained interviewer using a structured questionnaire (Gammon et al., 2004a; Steck et al., 2007; White et al., 2014); and vehicular traffic exposure was assessed by a validated historical geographic model (Beyea et al., 2006; Mordukhovich et al., 2015). Detailed LIBCSP PAH source assessment methods have been previously published (Gammon et al., 2004a; Mordukhovich et al., 2015; Steck et al., 2007; White et al., 2014). The PAH variable definitions, based on previous published associations with breast cancer, and total sample sizes used in the study reported here are described below.

Current active smoking (yes, no) was defined as smoking within the 12 months prior to the reference date, which was date of diagnosis for cases and date of identification for controls (n=1553 controls/1508 cases) (Gammon et al. 2004a). Participants were asked if they had lived with a smoking spouse to determine residential ETS exposure (yes, no) (n=1515 controls/1468 cases) (Gammon et al., 2004a). Frequency of grilled/smoked meat intake was assessed for each of six decades across the life course, or fewer depending on age at diagnosis (Steck et al., 2007). Lifetime intake was defined as the average servings consumed per year based on quantile distributions in the controls ($<33\%$ vs $\geq 33\%$ percentile or <55 servings/year, 55+ servings/year) (n=1515 controls/1468 cases) (Steck et al., 2007). Residential stove and fireplace use was defined as using a stove/fireplace in a Long Island residence for at

least 3 times per year and whether or not participants burned synthetic logs (yes, no) ($n=1541$ controls/1501 cases) (White et al., 2014). A geographic model estimated vehicular traffic exposure for the study participant's residence in 1995 by incorporating historical United States (U.S.) vehicular PAH emissions data, information on traffic and transportation patterns, Long Island, NY meteorological variables and pollutant dispersion factors (<95th percentile, ≥ 95th percentile) ($n=1334$ controls/1274 cases) (Mordukhovich et al., 2015). This cutpoint was previously found to best represent the association with breast cancer incidence (Mordukhovich et al., 2015).

As a sensitivity analysis, more refined exposure classifications for current active smoking (no current, current <20 pack-years, current 20+ pack-years), spouse ETS exposure in months (tertiles), grilled/smoked meat intake (tertiles) and vehicular traffic (<50th percentile, 50–75th percentile, 75–95th percentile, 95th percentile) were also evaluated.

2.3. Gene-specific promoter DNA methylation assessment

Promoter methylation status was measured in tumor tissue for a panel of 13 breast cancer-related genes [*adenomatous polyposis coli (APC), breast cancer 1, early onset (BRCA1), cyclin D2 (CCND2), E-Cadherin (CDH1), death-associated protein kinase 1 (DAPK1), estrogen receptor 1 (ESR1), glutathione S-transferase pi 1 (GSTP1), secretoglobin, family 3A, member 1 (HIN1), cyclin-dependent kinase inhibitor 2A (CDKN2A), progesterone receptor (PGR), retinoic acid receptor, beta (RARβ, Ras association domain family member 1 (RASSF1A)* and *twist homolog 1 (TWIST1)*]. These genes are known to play an important role in breast carcinogenesis and their promoter regions are frequently methylated in breast tumor tissues (Xu et al., 2011). Methylation at certain CpG sites may vary by biologic sample type (Jones, 2012) and the measurement of gene-specific methylation in the tumor tissue is more sensitive than measuring the same sites in circulating blood DNA (Brooks et al., 2009).

The methods used to determine gene-specific promoter methylation status have been previous published and are briefly described below (Xu et al., 2011; Xu et al., 2012). DNA was extracted from tumor blocks, as described in previously published methods (Xu et al., 2009). To determine methylation levels for *ESR1*, *PGR* and *BRCA1*, methylation-specific PCR was used (Liu et al., 2003; Xu et al., 2009). The gene was determined to be methylated or not methylated based on whether PCR product was obtained using methylation-specific primers. Thus, *ESR1, PGR* and *BRCA1* are dichotomous variables (methylated vs. unmethylated), as determined by the assay. Once it became available, the quantitative MethyLight assay was used to measure the methylation status of the remaining 10 genes (Eads et al., 1999; Eads et al., 2000a). Bisulfite-converted genomic DNA was amplified using a fluorescence-based, real-time quantitative PCR which results in a percentage methylated (Chomczynski and Sacchi, 1987; Gibson et al., 1996). Continuous values were dichotomized ($<4\%$, $\geq 4\%$ methylated) to be consistent with previous published reports by our study team and others (Eads et al., 2000a; Xu et al., 2011). This $\geq 4\%$ cutpoint for the MethyLight assay has been previously reported to distinguish between malignant and normal tissues and is indicative of repressed gene expression (Eads et al., 2000b; Ogino et al., 2006). Number of case samples completed and percent methylated for each promoter in LIBCSP have been previously reported ($n=765$–851 and percent methylation 3.6–62.9%) (Xu et al., 2011).

2.4. Global methylation assessment

Two complimentary, but independent, methods were used to assess global methylation levels in DNA extracted from blood samples, LINE-1 and LUMA (1055 cases/1101 controls) (Xu et al., 2012). The LINE-1 assay is a measure of methylation at repetitive elements as a proxy measure of overall global methylation and it is hypothesized that lower methylation at LINE-1 may indicate increased chromosomal instability and likelihood of mutations (Brennan and Flanagan, 2012). In contrast, the LUMA measure summarizes methylation levels (5-mC) at all "CCGG" sequences and thus, may be considered more representative of gene-specific methylation (Brennan and Flanagan, 2012).

The LINE-1 assay was completed using a prevalidated pyrosequencing assay to assess 4 CpG sites in the promoter of LINE-1 at EpigenDx (Worcester, MA, USA) as described previously (Xu et al., 2012). Methylation status at each of the 4 CpG loci was analyzed individually as a T/C single nucleotide polymorphism (SNP) using QCpG software (Qiagen). Methylation status data at all 4 loci were averaged to provide an overall percent 5-^{m}C status. The LUMA assay is expressed as a percentage obtained using the following equation (Bjornsson et al., 2008): methylation (%)$=((1-(\mathrm{HpaII}\sum G/\sum T)/(\mathrm{MspI}\sum G/\sum T)^{*}100))$.

2.5. Hormone receptor subtype

Breast cancer subtype for the first primary was defined by estrogen/progesterone receptor status (ER/PR) obtained from the medical record, and was available for 65.6% of cases ($n=990$) (Gammon et al., 2002).

2.6. Statistical analysis

All analyses were completed using SAS 9.3 (Cary, NC). To investigate whether the five PAH sources, considered separately, were associated with methylation levels, we used unconditional logistic regression to estimate odds ratios (ORs) and corresponding 95% confidence intervals (CIs). Multiplicative interaction was assessed by comparing multivariable models with and without cross-product terms to denote the interaction using an *a priori* alpha level of 0.05.

For examining gene-specific promoter methylation, we used a case-case approach to determine whether PAH source exposures were associated with methylation in tumor tissue. Tumor subtypes were defined as methylated versus non-methylated. If the gene-specific promoter sample size within a stratum was less than 5, it was no longer included in the analysis. We explored whether the association between sources of PAH and gene-specific promoter methylation varied by hormone receptor status by testing for a multiplicative interaction between the PAH source, gene-promoter methylation and ER/PR status of the tumor.

For examining global methylation, we first used a controls-only approach to assess whether PAH sources were associated with LINE-1 or LUMA measured in control blood samples. LINE-1 and LUMA levels were dichotomized based on the distribution in the controls as neither LINE-1 nor LUMA were normally distributed before or after natural log transformation. We subsequently utilized a case-control approach to assess whether the relationship between PAH sources and breast cancer incidence was modified by LUMA and/or LINE-1, on a multiplicative scale using both the continuous variable and the dichotomized variable. For the study reported here, global methylation assay results among cases are based on blood samples donated prior to chemotherapy ($n=79\%$). Additionally, in order to compare our results with previous research (Pirouzpanah et al., 2010; Tao et al., 2014), we conducted a sensitivity analysis to determine if LUMA was associated with wood-burning in the controls and if ever smoking was associated with gene-specific methylation in the tumor of cases.

Confounders were identified using a directed acyclic graph

(Glymour and Greenland, 2008) and included age at menarche (< 12, ≥ 12); parity (nulliparous, parous); lifetime alcohol intake (non-drinkers, < 15 g/day, 15–30 g/day, ≥ 30 g/day); education (high school graduate or less, some college, college graduate); income (< $34,999, $35,000-$69,999, ≥ $70,000); and the frequency matching factor, 5-year age group.

3. Results

3.1. Gene-specific promoter methylation in tumor tissue

Results for the associations between each individual source of PAH exposure and the 13 gene-specific methylation markers are shown in Tables 1–5, respectively. ORs greater than 1 indicate increased odds of methylation and ORs less than 1 indicate decreased odds of methylation. There were similarities in associations between the gene-specific DNA methylation measured in tumor tissue and the PAH sources (Tables 1–5). For example, ORs for *CCND2* methylation were reduced for residential ETS (Table 2; OR=0.65, 95%CI=0.44–0.96) and perhaps for high grilled/smoked meat intake (Table 1; OR=0.69, 95%CI=0.46–1.06), although confidence intervals for the latter included the null value. ORs for *RARβ* methylation were elevated in association with burning of synthetic logs (Table 4; OR=1.80, 95%CI=1.16–2.78) and for high grilled/smoked meat intake (Table 1; OR=1.39, 95%CI=0.94–2.08). ORs for *BRCA1* were reduced, indicating hypomethylation in association with synthetic logs use (OR=0.44, 95%CI=0.30–0.66), and perhaps with current active smoking (OR=0.74, 95%CI=0.52–1.07), although the confidence intervals for the latter included the null value (Tables 3 and 4, respectively).

Additionally, current active smoking was associated with lower odds of *DAPK* methylation (OR=0.53, 95%CI=0.28–0.99) (Table 3) although no other associations with *DAPK* were evident. Lower odds of *ESR1* methylation was observed in association with residential ETS (OR=0.74, 95%CI=0.56–0.99) (Table 2). Synthetic log use was associated with elevated odds of *HIN1* methylation (OR=2.14, 95%CI=1.34–3.42) and *CDH1* methylation (OR=2.26, 95%CI=1.06–4.79) (Table 4). Vehicular traffic was associated with higher odds of *TWIST1* methylation (OR=2.79, 95%CI=1.24–6.30) (Table 5).

Ever active smoking, while not included in our *a priori* exposures of interest, was additionally found to be associated with decreased odds of methylation of *ESR1* (OR=0.69, 95%CI 0.52–0.92) and *HIN1* (OR=0.66, 95%CI 0.48–0.91) (Appendix Table 1). We did not observe any evidence of effect measure modification of the association between PAH sources and gene-specific methylation by hormone receptor status of the tumor (data not shown).

3.2. Global methylation in peripheral blood

Synthetic log use in the home was associated with hypomethylation of LINE-1 in controls (OR=0.59, 95%CI=0.41–0.86) and suggestively associated with a pattern of hypomethylation in the LUMA assay (OR=0.75, 95%CI=0.52–1.09) (Appendix Table 2). None of the other PAH sources, including indoor wood burning in a stove or fireplace (data not shown), were associated with either LINE-1 or LUMA. We did not observe any evidence of effect measure modification of the association between the PAH sources and breast cancer incidence by global methylation markers (Appendix Tables 3 and 4), or tumor heterogeneity considering hormone receptor status of the tumor (data not shown) with either continuous or dichotomized global methylation variables.

3.3. Sensitivity analyses

For both global and gene-specific analyses, more refined exposure classifications for the PAH sources were considered. All results and conclusions remained the same (data not shown) and therefore the results for the dichotomized PAH exposure sources were included here as they best represented associations and functioned to maximize power.

4. Discussion

The sources of PAH exposure examined here were associated with a number of methylation sites in both the gene-specific tumor of cases and the blood of controls supporting the hypothesis that DNA methylation may be one of the potential biologic mechanisms for the association between PAH sources and breast cancer. We considered sources of PAH exposure, and classifications of exposure, based on our previous studies (Gammon et al., 2004a; Mordukhovich et al., 2015; Steck et al., 2007; White et al., 2014) and others (Fu et al., 2011; Gaudet et al., 2013; Hystad et al., 2015; Morabia 2002), with breast cancer incidence. Thus, many of the associations with DNA methylation observed among our population-based sample of women with breast cancer are biologically plausible and relevant for breast carcinogenesis. For example, we observed decreased methylation in the tumor of *CCND2*, an

Table 1
Odds of gene specific promoter methylation in tumor of cases in association with average lifetime intake of grilled/barbecued/smoked meat intake, LIBCSP 1996–1997[a].

	No active smoking			Ever active smoking		
Genes	**Unmethylated (*N*)**	**Methylated (*N*)**	**OR (95%CI)**	**Unmethylated (*N*)**	**Methylated (*N*)**	**OR (95%CI)**
ESR1	187	187	1.00(reference)	273	196	0.69(0.52, 0.92)
PGR	327	51	1.00(reference)	422	51	0.87(0.56, 1.35)
BRCA1	149	229	1.00(reference)	198	275	0.93(0.69, 1.24)
APC	186	166	1.00(reference)	227	221	1.04(0.78, 1.40)
CDKN2A	329	14	1.00(reference)	418	16	0.75(0.34, 1.64)
HIN1	107	223	1.00(reference)	177	258	0.66(0.48, 0.91)
RASSF1a	47	283	1.00(reference)	66	369	0.99(0.65, 1.53)
DAPK1	284	46	1.00 (reference)	373	62	0.96(0.62, 1.49)
GSTP1	233	97	1.00(reference)	319	116	0.84(0.60, 1.18)
CCND2	272	58	1.00(reference)	343	92	1.33(0.90, 1.98)
TWIST1	279	51	1.00(reference)	370	65	0.95(0.62, 1.46)
CDH1	311	19	1.00(reference)	410	25	0.85(0.44, 1.63)
RARβ	236	94	1.00(reference)	318	117	0.90(0.64, 1.26)

15–30 g/day, ≥ 30 g/day), education (high school graduate or less, some college, college or post-college), income (< $34,999,$35,000–69,999, ≥ $70,000) and the matching factor, 5-year age group.

[a] Adjusted for age at menarche (< 12, ≥ 12 years), parous (nulliparous, parous), lifetime alcohol intake (non-drinkers, < 15 g/day).

Table 2
Odds of gene specific promoter methylation in tumor of cases in association with long-term residential environmental tobacco smoke (ETS), LIBCSP 1996–1997[a].

	No residential ETS			Residential ETS		
Genes	**Unmethylated (*N*)**	**Methylated (*N*)**	**OR (95%CI)**	**Unmethylated (*N*)**	**Methylated (*N*)**	**OR (95%CI)**
ESR1	210	195	1.00(reference)	241	173	0.74(0.56, 0.99)
PGR	358	51	1.00(reference)	369	48	1.00(0.64, 1.56)
BRCA1	166	243	1.00(reference)	174	243	1.03(0.77, 1.39)
APC	203	183	1.00(reference)	199	192	1.01(0.75, 1.36)
CDKN2A	364	13	1.00(reference)	361	16	0.97(0.44, 2.14)
HIN1	130	226	1.00 (reference)	142	244	1.02(0.74, 1.41)
RASSF1a	48	308	1.00(reference)	63	323	0.83(0.53, 1.28)
DAPK1	309	47	1.00(reference)	329	57	1.05(0.67, 1.62)
GSTP1	255	101	1.00(reference)	281	105	0.91(0.65, 1.28)
CCND2	279	77	1.00(reference)	318	68	0.65 (0.44, 0.96)
TWIST1	298	58	1.00(reference)	329	57	0.89(0.58, 1.36)
CDH1	335	21	1.00(reference)	364	22	0.86(0.45, 1.67)
RARβ	254	102	1.00(reference)	283	103	0.86(0.61, 1.21)

15–30 g/day, ≥ 30 g/day), education (high school graduate or less, some college, college or post-college), income (< $34,999,$35,000–69,999, ≥ $70,000) and the matching factor, 5-year age group.

[a] Adjusted for age at menarche (< 12, ≥ 12 years), parous (nulliparous, parous), lifetime alcohol intake (non-drinkers, < 15 g/day).

oncogene, in association with residential ETS. Lower methylation at some CpGs in *CCND2* is correlated with increased mRNA expression in The Cancer Genome Atlas (TCGA) breast tumor data (Gao et al., 2013). Conversely, we observed increased methylation for *RARβ, HIN1* and *CDH1* in association with the sources of PAH exposure. Both *HIN1* and *CDH1* are tumor suppressor genes, and increased methylation levels are associated with decreased expression of these genes in TCGA data(Gao et al., 2013). Decreased expression of RARβ, a steroid hormone receptor that is important for maintaining normal cell growth and regulation, has also been found to play a role in breast carcinogenesis (Pirouzpanah et al., 2010). Thus, PAH sources may be both increasing expression of oncogenes and repressing expression of tumor suppressor and other genes important for normal cell functioning.

We observed that PAH sources were associated with DNA methylation in similar directions at certain gene promoter regions, despite differences in exposure source or even route of exposure. For example, we reported decreased ORs for methylation at *CCND2* for both residential ETS and grilled/smoked meat intake. This pattern of findings is supportive of similar biologic mechanisms across the exposure sources. We observed either increased or decreased ORs for methylation across different genes, which is consistent with other epidemiologic studies of active smoking in healthy individuals that have found associations with both increases and decreases in methylation that are site-specific (Zeilinger et al., 2013; Zhang et al., 2014). Similarly, a study in breast cancer cell lines found that treatment with the PAH compound benzo[a]pyrene (BaP) induces both hyper- and hypomethylation at different sites (Sadikovic and Rodenhiser, 2006). Although the mechanisms for the association between PAHs and DNA methylation are not completely understood, there are a number of possible mechanisms by which PAHs may disturb DNA methylation patterns resulting in both increases and reductions in methylation levels. There is evidence that a BaP metabolite, benzo(a)pyrene diolepoxide (BPDE), has enhanced binding to DNA at methylated CpG sites (Yoon et al., 2001) and that DNA methyltransferase bind to DNA lesions with high affinity when there is DNA damage such as that from carcinogenic adducts (James et al., 2003). Additionally, BPDE can inhibit the function of methyltransferase enzymes resulting in a loss of methylation (Wilson and Jones, 1984). It is also possible that methylation may be lost during the repair of carcinogenic adducts from PAH exposure via the nucleotide excision repair pathway (Holliday, 1979). Repair of these damaged regions could lead to hypomethylation if methylation is not regained prior to DNA replication (Sadikovic and Rodenhiser, 2006).

In addition to our findings with gene-specific methylation, exposure to burning synthetic logs was found to be associated with LINE-1 hypomethylation in the peripheral blood among our

Table 3
Odds of gene specific promoter methylation in tumor of cases in association with current active smoking, LIBCSP 1996–1997[a].

	No current active smoking			Current active smoking		
Genes	**Unmethylated (*N*)**	**Methylated (*N*)**	**OR (95%CI)**	**Unmethylated (*N*)**	**Methylated (*N*)**	**OR (95%CI)**
ESR1	369	309	1.00 (reference)	91	74	0.94(0.66, 1.35)
PGR	601	84	1.00 (reference)	148	18	0.94(0.54, 1.66)
BRCA1	269	416	1.00 (reference)	78	88	0.74(0.52, 1.07)
APC	333	306	1.00 (reference)	80	81	1.09(0.76, 1.57)
CDKN2A	598	24	1.00 (reference)	149	6	0.81(0.30, 2.14)
HIN1	224	338	1.00 (reference)	60	93	0.81(0.55, 1.20)
RASSF1a	86	526	1.00 (reference)	27	126	0.70(0.42, 1.15)
DAPK1	517	95	1.00 (reference)	140	13	0.53(0.28, 0.99)
GSTP1	329	173	1.00 (reference)	113	40	0.82(0.53, 1.24)
CCND2	491	121	1.00 (reference)	124	29	1.12(0.69, 1.81)
TWIST1	517	95	1.00 (reference)	132	21	0.96(0.56, 1.65)
CDH1	578	34	1.00 (reference)	143	10	1.07(0.50, 2.34)
RARβ	442	170	1.00 (reference)	112	41	0.94(0.62, 1.44)

15–30 g/day, ≥ 30 g/day), education (high school graduate or less, some college, college or post-college), income (< $34,999,$35,000–69,999, ≥ $70,000) and the matching factor, 5-year age group.

[a] Adjusted for age at menarche (< 12, ≥ 12 years), parous (nulliparous, parous), lifetime alcohol intake (non-drinkers, < 15 g/day).

Table 4
Odds of gene specific promoter methylation in tumor of cases in association with residential burning of synthetic logs, LIBCSP 1996–1997[a].

	No synthetic log use			Synthetic log use		
Genes	**Unmethylated (*N*)**	**Methylated (*N*)**	**OR (95%CI)**	**Unmethylated (*N*)**	**Methylated (*N*)**	**OR (95%CI)**
ESR1	381	325	1.00 (reference)	79	56	0.80(0.55, 1.19)
PGR	625	87	1.00 (reference)	121	15	0.82(0.45, 1.50)
BRCA1	270	442	1.00 (reference)	75	62	0.44(0.30, 0.66)
APC	347	322	1.00 (reference)	65	64	1.04(0.70, 1.55)
CDKN2A	624	25	1.00 (reference)	121	5	1.03(0.37, 2.87)
HIN1	256	394	1.00 (reference)	28	86	2.14(1.34, 3.42)
RASSF1a	101	549	1.00 (reference)	12	102	1.68(0.87, 3.24)
DAPK1	559	91	1.00 (reference)	97	17	1.13(0.63, 2.03)
GSTP1	473	177	1.00 (reference)	78	36	1.30(0.83, 2.03)
CCND2	523	127	1.00 (reference)	91	23	1.20(0.72, 2.03)
TWIST1	551	99	1.00 (reference)	97	17	1.03(0.58, 1.85)
CDH1	617	33	1.00 (reference)	103	11	2.26(1.06, 4.79)
RARβ	480	170	1.00 (reference)	73	41	1.80(1.16, 2.78)

15–30 g/day, ≥ 30 g/day), education (high school graduate or less, some college, college or post-college), income (< $34,999,$35,000–69,999, ≥ $70,000) and the matching factor, 5-year age group.

[a] Adjusted for age at menarche (< 12, ≥ 12 years), parous (nulliparous, parous), lifetime alcohol intake (non-drinkers, < 15 g/day).

population-based sample of women without breast cancer. LINE-1 hypomethylation in the peripheral blood, hypothesized to indicate chromosomal instability and increased likelihood for mutations, has been found to be associated with breast cancer in a prospective cohort (Deroo et al., 2014), although such an association was not observed in the LIBCSP (Xu et al., 2012). BaP has also been found to decrease global methylation *in vitro* (Wilson and Jones, 1983). In contrast to our null findings reported here for the LIBCSP population, a study conducted in Warsaw, Poland reported an inverse association between indoor air pollution measured by wood and coal burning with peripheral blood global methylation measured by LUMA (Tao et al., 2014). However, the investigators of the Polish study did not consider synthetic log burning which is likely less commonly used in their study population.

Several issues regarding our PAH source measures may impact our study interpretation. We selected the PAH source variables included here because each was previously reported to be most relevant to breast cancer incidence in our study population (Gammon et al., 2004a; Mordukhovich et al., 2015; Steck et al., 2007; White et al., 2014). However, other PAH source classifications (e.g., ever or former smokers) and other time frames of exposures may also be relevant for methylation alterations (Shenker et al., 2013). Our study design limited our ability to isolate specific PAH carcinogens, yet the PAH carcinogens found in each exposure source are known to vary (Boström et al., 2002). Also, the PAH exposure sources considered here contain other chemicals (IARC 2010). These variations across exposure sources, however, could be relevant to DNA methylation changes and may be reasons for some discrepancy in patterns across methylated sites among studies and PAH sources (Ruiz-Hernandez et al., 2015).

In our population-based sample of women, who were ambiently exposed to PAH from multiple sources, we were able to consider both gene-specific and global methylation with a number of PAH exposure sources, specifically focusing on those which our study team has previously found to be associated with breast cancer. We were also able to evaluate potential heterogeneity by hormone receptor subtype, as methylation level at a given locus may be particularly associated with specific breast cancer tumor subtypes over others (Avraham et al., 2014). Consideration of tumor subtype did not alter our conclusions, possibly because the LIBCSP study population is predominately women with hormone receptor positive tumors and therefore, power to assess associations in receptor negative tumors was limited (Gammon et al., 2004a).

Few previous studies have considered associations between tobacco smoke and DNA methylation in breast cancer tissue; one study found ever smoking to be associated with *ESR1* hypermethylation in breast tissue of Iranian women (n = 137)

Table 5
Odds of gene specific promoter methylation in tumor of cases in association with vehicular traffic exposure,LIBCSP 1996–1997[a].

	Low vehicular traffic (< 95th percentile)			High vehicular traffic (≥ 95th percentile)		
Genes[b]	**Unmethylated (*N*)**	**Methylated (*N*)**	**OR (95%CI)**	**Unmethylated (*N*)**	**Methylated (*N*)**	**OR (95%CI)**
ESR1	354	310	1.00(reference)	24	18	0.74(0.38, 1.45)
PGR	594	77	1.00(reference)	37	5	0.90(0.30, 2.65)
BRCA1	273	398	1.00(reference)	20	22	0.72(0.38, 1.40)
APC	327	307	1.00(reference)	18	18	1.11(0.54, 2.25)
HIN1	221	376	1.00(reference)	13	25	1.35(0.65, 2.80)
RASSF1a	88	509	1.00(reference)	8	30	0.60(0.26, 1.42)
DAPK1	515	82	1.00(reference)	33	5	0.94(0.34, 2.57)
GSTP1	431	166	1.00(reference)	26	12	1.18(0.56, 2.15)
CCND2	482	115	1.00(reference)	32	6	0.66(0.24, 1.78)
TWIST1	520	77	1.00(reference)	28	10	2.79(1.24, 6.30)
RARβ	433	164	1.00(reference)	32	6	0.49(0.20, 1.23)

15–30 g/day, ≥ 30 g/day), education (high school graduate or less, some college, college or post-college), income (< $34,999,$35,000–69,999, ≥ $70,000) and the matching factor, 5-year age group.

[a] Adjusted for age at menarche (< 12, ≥ 12 years), parous (nulliparous, parous), lifetime alcohol intake (non-drinkers, < 15 g/day).

[b] *CDKN2A* and *CDH1* had cell sizes less than 5 and odds ratios were not estimated.

(Pirouzpanah et al., 2010), which we did not observe when we considered ever active smoking, which we specifically examined in order to try and replicate this finding. Differences in active smoking exposure history due to geographic and cultural difference between studies may, at least in part, explain this discrepancy. Methylation levels may reflect both long-term and recent exposures, so it is biologically plausible for variables reflecting different time periods to both be relevant (Shenker et al., 2013). Ever active smoking and current active smoking were associated with different methylation markers in this study, suggesting that methylation may change after stopping smoking (Tsaprouni et al., 2014) or that current smokers may represent those with a higher intensity or longer duration of tobacco use and thus may have different methylation patterns for that reason. Other studies have focused on white blood cell methylation in healthy individuals, although inconsistent associations between air pollution and smoking with both gene-specific and global methylation have been observed (Terry et al., 2011). However, it is unclear whether global methylation measures accurately reflect that of the target tissue (Brooks et al., 2009). Thus, as compared with previous investigations, our study expands the specific research questions addressed and the study approach, by utilizing a larger population-based study population as well as broadening the scope of the relevant PAH sources and DNA methylation targets considered.

We used a panel of *a priori* genes, and thus, this study cannot rule out that there are other methylation sites which could be relevant to PAHs and breast cancer. We did not adjust for multiple comparisons; all comparisons made were driven by biologically plausible hypotheses and we did not rely on statistical significance for interpretation of measures of association and focused on evaluating trends (Savitz and Olshan, 1995). However, we cannot rule out that some of these associations may be due to chance, because in our study there are some instances of low prevalence of methylated genes and small within stratum sizes that produced imprecise estimates and may have resulted in over fitting of the models. Regarding generalizability, the women in the LIBCSP are predominately white and postmenopausal (Gammon et al., 2002), and therefore our results may not be applicable to all women.

In this first population-based study to report on gene-specific methylation in association with current active smoking, residential ETS, synthetic log burning, grilled/smoked meat intake and vehicular traffic, we identified biologically plausible associations with aberrant DNA methylation in the tumor tissue. DNA methylation represents a potential biologic mechanism for environmental chemicals, such as PAH, to influence breast cancer risk.

Acknowledgments

Supported in part by Grants from the: National Institutes of Health (R25 CA57726, UO1CA/ES66572, R01CA109753, 3R01CA109753-04S1, P30CA013696, CA094061, P30ES009089 and P30ES10126); and Department of Defense (BC031746 and W81XWH-06-1-0298).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2015.11.033.

References

Avraham, A., Cho, S.S., Uhlmann, R., Polak, M.L., Sandbank, J., Karni, T., et al., 2014. Tissue specific DNA methylation in normal human breast epithelium and in breast cancer. Plos One 9, e91805.

Beyea, J., Hatch, M., Stellman, S.D., Santella, R.M., Teitelbaum, S.L., Prokopczyk, B., et al., 2006. Validation and calibration of a model used to reconstruct historical exposure to polycyclic aromatic hydrocarbons for use in epidemiologic studies. Environ. Health Perspect. 114, 1053–1058.

Bjornsson, H.T., Sigurdsson, M.I., Fallin, M.D., Irizarry, R.A., Aspelund, T., Cui, H., et al., 2008. Intra-individual change over time in DNA methylation with familial clustering. JAMA 299, 2877–2883.

Bollati, V., Baccarelli, A., 2010. Environmental epigenetics. Heredity 105, 105–112.

Boström, C.E., Gerde, P., Hanberg, A., Jernstrom, B., Johansson, C., Kyrklund, T., et al., 2002. Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environ. Health Perspect. 3, 451–488.

Brennan, K., Flanagan, J.M., 2012. Is there a link between genome-wide hypomethylation in blood and cancer risk? Cancer Prev. Res. 5, 1345–1357.

Brooks, J., Cairns, P., Zeleniuch-Jacquotte, A., 2009. Promoter methylation and the detection of breast cancer. Cancer Causes Control 20, 1539–1550.

Cho, Y.H., Shen, J., Gammon, M.D., Zhang, Y.J., Wang, Q., Gonzalez, K., et al., 2012. Prognostic significance of gene-specific promoter hypermethylation in breast cancer patients. Breast Cancer Res. Treat. 131, 197–205.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162, 156–159.

Deroo, L.A., Bolick, S.C., Xu, Z., Umbach, D.M., Shore, D., Weinberg, C.R., et al., 2014. Global DNA methylation and one-carbon metabolism gene polymorphisms and the risk of breast cancer in the Sister Study. Carcinogenesis 35, 333–338.

Duan, H., He, Z., Ma, J., Zhang, B., Sheng, Z., Bin, P., et al., 2013. Global and MGMT promoter hypomethylation independently associated with genomic instability of lymphocytes in subjects exposed to high-dose polycyclic aromatic hydrocarbon. Arch. Toxicol. 87, 2013–2022.

Eads, C.A., Danenberg, K.D., Kawakami, K., Saltz, L.B., Danenberg, P.V., Laird, P.W., 1999. CpG island hypermethylation in human colorectal tumors is not associated with DNA methyltransferase overexpression. Cancer Res. 59, 2302–2306.

Eads, C.A., Danenberg, K.D., Kawakami, K., Saltz, L.B., Blake, C., Shibata, D., et al., 2000a. MethyLight: a high-throughput assay to measure DNA methylation. Nucleic Acids Res. 28, E32.

Eads, C.A., Lord, R.V., Kurumboor, S.K., Wickramasinghe, K., Skinner, M.L., Long, T.I., et al., 2000b. Fields of aberrant CpG island hypermethylation in Barrett's esophagus and associated adenocarcinoma. Cancer Res. 60, 5021–5026.

Fu, Z., Deming, S.L., Fair, A.M., Shrubsole, M.J., Wujcik, D.M., Shu, X.O., et al., 2011. Well-done meat intake and meat-derived mutagen exposures in relation to breast cancer risk: the Nashville Breast Health Study. Breast Cancer Res. Treat. 129, 919–928.

Gammon, M.D., Neugut, A.I., Santella, R.M., Teitelbaum, S.L., Britton, J.A., Terry, M.B., et al., 2002. The Long Island Breast Cancer Study Project: description of a multi-institutional collaboration to identify environmental risk factors for breast cancer. Breast Cancer Res. Treat. 74, 235–254.

Gammon, M.D., Eng, S.M., Teitelbaum, S.L., Britton, J.A., Kabat, G.C., Hatch, M., et al., 2004a. Environmental tobacco smoke and breast cancer incidence. Environ. Res. 96, 176–185.

Gammon, M.D., Sagiv, S.K., Eng, S.M., Shantakumar, S., Gaudet, M.M., Teitelbaum, S. L., et al., 2004b. Polycyclic aromatic hydrocarbon-DNA adducts and breast cancer: a pooled analysis. Arch. Environ. Health 59, 640–649.

Gao, J., Aksoy, B.A., Dogrusoz, U., Dresdner, G., Gross, B., Sumer, S.O., et al., 2013. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci. Signal. 6, pl1.

Gaudet, M.M., Gapstur, S.M., Sun, J., Diver, W.R., Hannan, L.M., Thun, M.J., 2013. Active smoking and breast cancer risk: original cohort data and meta-analysis. J. Natl. Cancer Inst. 105, 515–525.

Gibson, U.E., Heid, C.A., Williams, P.M., 1996. A novel method for real time quantitative RT-PCR. Genome Res. 6, 995–1001.

Glymour, M.M., Greenland, S., 2008. Causal diagrams, Modern Epidemiology, 3rd ed. Lippincott Williams & Wilkins, Philadelphia, PA.

Hecht, S.S., 2002. Tobacco smoke carcinogens and breast cancer. Environ. Mol. Mutagen. 39, 119–126.

Holliday, R., 1979. A new theory of carcinogenesis. Br. J. Cancer 40, 513.

Hystad, P., Villeneuve, P.J., Goldberg, M.S., Crouse, D.L., Johnson, K., 2015. Exposure to traffic-related air pollution and the risk of developing breast cancer among women in eight Canadian provinces: a case-control study. Environ. Int. 74, 240–248.

IARC, 2010. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. (IARC monographs on the evaluation of carcinogenic risks to humans / World Health Organization, International Agency for Research on Cancer). 1017-1606.IARC Working Group on the Evaluation of Carcinogenic Risks to Humans.

James, S.J., Pogribny, I.P., Pogribna, M., Miller, B.J., Jernigan, S., Melnyk, S., 2003. Mechanisms of DNA damage, DNA hypomethylation, and tumor progression in the folate/methyl-deficient rat model of hepatocarcinogenesis. J. Nutr. 133, 3740S–3747S.

Jones, P.A., 2012. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat. Rev. Genet. 13, 484–492.

Liu, Z.J., Maekawa, M., Horii, T., Morita, M., 2003. The multiple promoter methylation profile of PR gene and ERalpha gene in tumor cell lines. Life Sci. 73, 1963–1972.

Morabia, A., Bernstein, M., Heritier, S., Khatchatrian, N., 1996. Relation of breast cancer with passive and active exposure to tobacco smoke. Am. J. Epidemiol.,

143.
Morabia, A., 2002. Smoking (active and passive) and breast cancer: epidemiologic evidence up to June 2001. Environ. Mol. Mutagen. 39, 89–95.
Mordukhovich, I., Beyea, J., Herring, A.H., Hatch, M., Stellman, S.D., Teitelbaum, S., et al., 2015. Exposure to traffic-related polycyclic aromatic hydrocarbons and breast cancer risk. Environ. Health Perspect. (Epub ahead of print).
Nie, J., Beyea, J., Bonner, M.R., Han, D., Vena, J.E., Rogerson, P., et al., 2007. Exposure to traffic emissions throughout life and risk of breast cancer: the Western New York Exposures and Breast Cancer (WEB) study. Cancer Causes Control. 18, 947–955.
Ogino, S., Kawasaki, T., Brahmandam, M., Cantor, M., Kirkner, G.J., Spiegelman, D., et al., 2006. Precision and performance characteristics of bisulfite conversion and real-time PCR (MethyLight) for quantitative DNA methylation analysis. J. Mol. Diagn. JMD 8, 209–217.
Pirouzpanah, S., Taleban, F.A., Atri, M., Abadi, A.R., Mehdipour, P., 2010. The effect of modifiable potentials on hypermethylation status of retinoic acid receptor-beta2 and estrogen receptor-alpha genes in primary breast cancer. Cancer Causes Control. 21, 2101–2111.
Ruiz-Hernandez, A., Kuo, C.-C., Rentero-Garrido, P., Tang, W.-Y., Redon, J., Ordovas, J. M., et al., 2015. Environmental chemicals and DNA methylation in adults: a systematic review of the epidemiologic evidence. Clin. Epigenetics 7, 55.
Sadikovic, B., Rodenhiser, D.I., 2006. Benzopyrene exposure disrupts DNA methylation and growth dynamics in breast cancer cells. Toxicol. Appl. Pharmacol. 216, 458–468.
Savitz, D.A., Olshan, A.F., 1995. Multiple comparisons and related issues in the interpretation of epidemiologic data. Am. J. Epidemiol. 142, 904–908.
Shenker, N.S., Ueland, P.M., Polidoro, S., van Veldhoven, K., Ricceri, F., Brown, R., et al., 2013. DNA methylation as a long-term biomarker of exposure to tobacco smoke. Epidemiology 24, 712–716.
Steck, S.E., Gaudet, M.M., Eng, S.M., Britton, J.A., Teitelbaum, S.L., Neugut, A.L., et al., 2007. Cooked meat and risk of breast cancer-Lifetime versus recent dietary intake. Epidemiology 18, 373–382.
Tao, M.H., Zhou, J., Rialdi, A.P., Martinez, R., Dabek, J., Scelo, G., et al., 2014. Indoor air pollution from solid fuels and peripheral blood DNA methylation: findings from a population study in Warsaw, Poland. Env. Res. 134, 325–330.
Terry, M.B., Zhang, F.F., Kabat, G., Britton, J.A., Teitelbaum, S.L., Neugut, A.I., et al., 2006. Lifetime alcohol intake and breast cancer risk. Ann. Epidemiol. 16, 230–240.
Terry, M.B., Delgado-Cruzata, L., Vin-Raviv, N., Wu, H.C., Santella, R.M., 2011. DNA methylation in white blood cells: association with risk factors in epidemiologic studies. Epigenetics Off. J. DNA Methylation Soc. 6, 828–837.
Tsaprouni, L.G., Yang, T.P., Bell, J., Dick, K.J., Kanoni, S., Nisbet, J., et al., 2014. Cigarette smoking reduces DNA methylation levels at multiple genomic loci but the effect is partially reversible upon cessation. Epigenetics Off. J. DNA Methylation Soc. 9, 1382–1396.
White, A.J., Teitelbaum, S., Stellman, S.D., Beyea, J., Steck, S.E., Mordukhovich, I., et al., 2014. Indoor air pollution exposure from use of indoor stoves and fireplaces in association with breast cancer: a case-control study. Environ. Health *13*, 108.
Wilson, V.L., Jones, P.A., 1983. Inhibition of DNA methylation by chemical carcinogens in vitro. Cell 32, 239–246.
Wilson, V.L., Jones, P.A., 1984. Chemical carcinogen-mediated decreases in DNA 5-methylcytosine content of BALB/3T3 cells. Carcinogenesis 5, 1027–1031.
Xu, X., Gammon, M.D., Zhang, Y., Bestor, T.H., Zeisel, S.H., Wetmur, J.G., et al., 2009. BRCA1 promoter methylation is associated with increased mortality among women with breast cancer. Breast Cancer Res. Treat. 115, 397–404.
Xu, X., Gammon, M.D., Jefferson, E., Zhang, Y., Cho, Y.H., Wetmur, J.G., et al., 2011. The influence of one-carbon metabolism on gene promoter methylation in a population-based breast cancer study. Epigenetics: official journal of the DNA Methylation. Society 6, 1276–1283.
Xu, X., Gammon, M.D., Hernandez-Vargas, H., Herceg, Z., Wetmur, J.G., Teitelbaum, S.L., et al., 2012. DNA methylation in peripheral blood measured by LUMA is associated with breast cancer in a population-based study. FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol. 26, 2657–2666.
Yoon, J.-H., Smith, L.E., Feng, Z., Tang, M.-s, Lee, C.-S., Pfeifer, G.P., 2001. Methylated CpG dinucleotides are the preferential targets for G-to-T transversion mutations induced by benzo [a] pyrene diol epoxide in mammalian cells similarities with the p53 mutation spectrum in smoking-associated lung cancers. Cancer Res. 61, 7110–7117.
Zeilinger, S., Kuhnel, B., Klopp, N., Baurecht, H., Kleinschmidt, A., Gieger, C., et al., 2013. Tobacco smoking leads to extensive genome-wide changes in DNA methylation. Plos One 8, e63812.
Zhang, Y., Yang, R., Burwinkel, B., Breitling, L.P., Brenner, H., 2014. F2RL3 methylation as a biomarker of current and lifetime smoking exposures. Environ. Health Perspect. 122, 131–137.