Systematic Review

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Epigenomics

Is breast cancer a result of epigenetic responses to traffic-related air pollution? A review of the latest evidence

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Environmental toxicants can exert adverse health effects via epigenetic regulation. We conducted a review of studies assessing traffic-related air pollution (TRAP) exposure and breast cancer (BC) risk, and the evidence for epigenetic mediation. 14 epidemiological studies demonstrated associations between TRAP exposure and BC risk, in which a total of 26 comparisons were assessed. 11 of these comparisons reported a positive association; whereas 15 comparisons were negative. Five publications linked TRAP exposure to epigenetic alterations in genes that may be related to BC risk. One animal study provided evidence of TRAP-treatment inducing breast tumorigenesis. Associations between TRAP components polycyclic aromatic hydrocarbons (PAH) and nitrogen dioxide (NO_2) and BC risk were more consistent. While evidence for epigenetic regulation remains limited, polycyclic aromatic hydrocarbons (PAH) and nitrogen dioxide (NO_2) exposures may alter methylation of breast tumorigenic genes (e.g., *EPHB2, LONP1*). Future epigenomic studies with environmental measures are needed to interrogate the relationship between TRAP and BC risk.

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Breast cancer (BC) is the most common cancer among women, leading to high rates of morbidity and mortality [1]. The high incidence of BC remains only partially explained by established risk factors such as age, family history, BC susceptibility genes, lack of breastfeeding, alcohol intake, cigarette smoking and weight gain in adulthood [2–4], and by greater BC screening [5]. Population-based cancer registry data have identified significant increases in incidence over relatively recent times, including the last three decades, particularly among young (ages 25–39 years) women [6,7]. An additional explanation for these high rates is that environmental causes and their associated mechanisms need to be considered [8]. In particular, many studies suggest that early (i.e., prenatal) exposures and during other time windows (i.e., puberty, pregnancy, lactation and menopause) when the breast cells are rapidly dividing may alter BC susceptibility [6,7,9–12].

Exposure to air pollution is an established risk factor for lung cancer [13]. Airborne emission sources from traffic in particular have been classified as carcinogens by the International Agency for Research on Cancer (IARC) [14]. However, emerging data are beginning to suggest that higher concentrations of traffic emissions are associated with elevated BC risk as well [15]. Traffic-related air pollution (TRAP) contains a mixture of several compounds, such as gaseous pollutants (carbon monoxide, nitrogen dioxide [NO_2], sulfur dioxide), particulate matter (PM), metals and organic compounds including benzene and polycyclic aromatic hydrocarbons (PAH) [16]. Some components of TRAP, including PAH, may act as endocrine disruptors, suggesting possible mechanistic links

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in breast carcinogenesis [14]. These, too, may be more deleterious to breast tissue during the prenatal, pubertal and pregnancy windows of susceptibility (WOS) to environmental toxicants when the breast tissue is changing in form and function [17–23].

Environmental toxicants can exert their effects through alteration of epigenetic regulatory mechanisms. By definition, these mechanisms refer to DNA methylation, post-translational modifications of histone, production of noncoding RNAs and other events that alter DNA accessibility and chromatin structure to regulate gene transcription, without altering the DNA sequence [24–28]. Growing evidence suggests that epigenetic regulation and its effects on gene transcription may play key intermediary steps between environmental exposures during critical time WOS and BC risk [29–34].

Methods

In order to conduct a systematic review of the evidence addressing whether TRAP may induce BC through epigenetic regulatory mechanisms, we conducted a PUBMED search using the following key words: vehicular traffic exposure AND breast cancer AND/OR epigenetic/methylation; particulate matter (PM) 2.5 AND breast cancer AND/OR epigenetic/methylation; PM10 AND breast cancer AND/OR epigenetic/methylation; PAH (polycyclic aromatic hydrocarbons) AND breast cancer AND/OR epigenetic/methylation; nitrogen dioxide (NO_2) AND breast cancer AND/OR epigenetic/methylation; nitrogen oxide (NOx) AND breast cancer AND/OR epigenetic/methylation. We included all relevant articles that matched the searched keywords in the article title and abstract. We excluded manuscripts prior to year 2000, and those not written in the English language. We included two additional studies related to the topic that were cited in articles we searched but missed the key word search criteria. In this review we found 14 epidemiological studies that assessed associations between TRAP exposure and BC risk, in which a total of 26 comparisons were reported. 11 of these comparisons reported a positive association and 15 comparisons were negative. Five publications linked TRAP exposure to epigenetic alterations in genes that may be related to BC risk. One animal study provided evidence of TRAP-treatment inducing breast tumorigenesis.

Below, and in Table 1, we summarize the research links that address two questions: Does exposure to TRAP associate with the development of breast cancer? and Is BC a result of epigenetic responses to TRAP? We consider both estimates and direct measures of traffic-related specific components (PM, PAH, NO_2 and NOx), and review findings that focused on specific time periods of exposure. We conclude with perspectives on the outstanding research gaps and future areas of investigation for these prevalent exposures and to discern their effects on a highly prevalent cancer via epigenetic regulation.

Studies examining vehicular traffic measures

One approach to measuring traffic exposure in BC studies has been to use self-reported data from questionnaires [32–36]. For example, self-reported childhood (under age of 14 years) residential exposure to TRAP based on recalled proximity of childhood residence to major roads was not consistently associated with breast cancer, but aspects of road type (e.g., presence of a median or barrier dividing the road) was associated with increased BC risk:hazard ratio [HR]: 1.4; 95% CI: 1.0–1.9) [36]. However, these recalled indicators of exposure could be misclassified; even if nondifferential, the extent of exposure may be inaccurate. Studies that measured exposure to TRAP based on the extent of vehicular traffic at the nearest cross-street found that those who reportedly lived near very high vehicular traffic ($\geq$3 lanes) during childhood demonstrated greater risk of BC compared with those who lived near less vehicular traffic (adjusted HR [aHR]) $\geq$ 3 lanes: 1.4; 95% CI: 1.0–1.9 vs aHR $<$ 3 lanes: 1.1; 95% CI: 0.9–1.3) [36]. However, an increased incidence rate ratio of BC was not observed among participants in the Danish Diet Cancer and Health cohort that enrolled from 1993 to 1997 and were followed through 2006, when using major street within 50 m as a marker of exposure to traffic (relative risk [RR]: 0.98; 95% CI: 0.78–1.22) [37].

Other epidemiological studies have measured childhood exposure by linking recalled childhood addresses to external sources of traffic data. These studies have the advantage of requiring knowledge of residential history without relying on individuals to recall features of the roads near their homes. For example, the Western New York Exposure and Breast (WEB) cancer case–control study examined BC risk in relation to traffic emissions estimated throughout life using a geographic location-based model [34]. Participants were women with primary histologically confirmed breast cancer, aged 35–79 years. Estimates were retrospectively calculated for traffic emission in women at menarche, their first birth, and 10 and 20 years before enrollment. WEB reported that higher exposure to traffic estimates at menarche was associated with higher odds of premenopausal BC (odds ratio [OR]: 2.31; 95% CI: 1.03–5.17) [34]. On the other hand, they did not find any significant associations between higher traffic estimates

Table 1. Summary of the articles assessing the association between TRAP exposure, possible epigenetic links, and the development of breast cancer.

Pollutants	Exposure assessment	Window of susceptibility	Population	TRAP breast cancer findings	Epigenetic breast cancer findings	Ref.
Vehicular traffic	Self-reported questionnaire	Childhood <14 years	35–74 year old whose sisters had BC (Sister study)	Increased BC risk: HR 1.4 (95% CI: 1.0–1.9)	–	[36]
	Geographic traffic model based on geocoded residential addresses	Menarche	35–79 year old with BC (WEB study)	Higher odds for pre-menopausal BC: OR 2.31 (95% CI: 1.03–5.17)	–	[34]
				–	Higher methylation of SYK: OR 2.37 (95% CI: 1.05–5.33)	[33]
		First birth		No association with pre-menopausal BC: OR 1.27 (95% CI: 0.48–3.32)	–	[34]
				–	Lower methylation of Cyclin D2: OR 0.40 (95% CI: 0.17–0.93)	[33]
		10 years before enrollment		No association with pre-menopausal BC: OR 1.58 (95% CI: 0.71–3.54)	–	[34]
				–	Lower methylation of Cyclin D2: OR 0.48 (95% CI: 0.26–0.89)	[33]
		20 years before enrollment		No association with pre-menopausal BC: OR 1.37 (95% CI: 0.64–2.92)	–	[34]
	Urban vs rural address	Prenatal	39–66 year old (E3N cohort)	Increased BC risk: HR 1.11 (95% CI: 1.05–1.18)	–	[38]
		Adulthood		No increase in BC risk: HR 1.04 (95% CI: 0.98–1.10)		
	GIS	Adulthood	50–64 year old with no history of BC	No association with BC: RR 0.98 (95% CI: 0.78–1.22)	–	[37]
Particulate matter	Geospatial analyses	3 years before diagnosis	50–69 year old with BC	Higher risk of BC death upper 3 quartiles of exposure: HR 2nd quartile 1.82 (95%CI: 1.15 – 2.89); HR 3rd quartile 1.73 (95% CI: 1.12–2.67); and HR 4th quartile 1.72 (95% CI: 1.08–2.75) vs 1st quartile	–	[42]
	Geographic traffic model based on geocoded residential addresses	Birth	35–79 year old with BC (WEB study)	No association with post-menopausal BC: OR 2.42 (95% CI: 0.97–6.09)	–	[41]
		Menarche		No association with post-menopausal BC: OR 1.45 (95% CI: 0.74–2.87)		
	Land-use regression	Adulthood	35–74 year old whose sisters had BC (Sister study)	No association with BC: PM2.5 – HR 1.03 (95% CI: 0.96 –1.11); PM10 – HR 0.99 (95% CI: 0.98–1.00)	–	[45]
	Geocoded residential addresses	3 years before diagnosis	>44 year old nurses with BC (Danish Nurses Cohort)	No association with BC: PM2.5 – HR 0.93 (95% CI: 0.82–1.05); PM10 – HR 1.02 (95% CI: 0.94–1.10)	–	[46]
	Geocoded residential addresses	2 years before diagnosis	25–42 year old with no history of BC (Nurses Health Study II)	No association with BC risk: PM2.5 – HR 0.90 (95% CI: 0.79–1.03); PM10 – HR 1.00 (95% CI: 0.93–1.07)	–	[47]
	Land-use regression	Adulthood	Middle aged with history of BC (EPIC-Italy and EPIC-Dutch cohort)	–	EPIC Dutch – No altered methylation of BC genes	[48]
					EPIC Italy – Lower methylation of PES1 (β: 0.22, p: 1.47e–7)	

All cohorts were comprised entirely of female individuals.

BC: Breast cancer; B[a]P: Benzo[a]pyrene; CYP8: Putative cytochrome p450; EPIC: European Prospective Investigation into Cancer and Nutrition; E3N: Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale; EPHB2: Ephrin type-B receptor 2; GIS: Geographic information system; HIBADH: 3-Hydroxyisobutyrate dehydrogenase; HR: Hazard ratio; LIBCSP: Long Island Breast Cancer study project; LonP1: Lon Peptidase 1; OR: Odds ratio; PBMNC: Peripheral blood mononuclear cell; RARβ: Retinoic acid receptor β; SLC25A28: Solute Carrier Family 25 Member 28; SYK: Spleen tyrosine kinase; TSC2: Tuberous sclerosis complex 2; TSP: Total suspended particle; TRAP: Traffic-related air pollutant; WEB: Western New York exposure and Breast Cancer study.

Table 1. Summary of the articles assessing the association between TRAP exposure, possible epigenetic links, and the development of breast cancer (cont.).

Pollutants	Exposure assessment	Window of susceptibility	Population	TRAP breast cancer findings	Epigenetic breast cancer findings	Ref.
Polycyclic aromatic hydrocarbons	Experimental B[a]P	Adolescence (30 day old)	Female rats	96.7% of those exposed developed mammary tumors with increased proliferation and invasion vs 26.7% among controls	–	[59]
	Geocoded residential addresses modeled with traffic patterns, meteorological data, pollution dispersion factors	Adulthood	Middle aged with BC (LIBCSP)	B[a]P exposure in the top 5% associated with ER-/PR- BC: OR 2.09 (95% CI: 1.08–4.06), but not ER+/PR+ BC: OR 0.74 (95% CI: 0.40–1.38)	–	[64]
	PAH–DNA adducts in blood			Highest quantile OR 1.51 (95%CI: 1.04–2.20) vs lowest quantile OR 1.13 (95% CI: 0.71–1.81) associated with BC	–	[62]
					No association with PBMNC global methylation	[66]
	PAH–DNA adducts in breast tumor			–	Higher methylation of RARβ (OR 2.69 95% CI: 1.02–7.12)	
	Geographic traffic model based on geocoded residential addresses	Menarche	35–79 year old with BC (WEB study)	Higher odds for pre-menopausal BC in 2nd quartile: OR 4.89 (95% CI: 1.34–17.83), 3rd quartile: OR 6.96 (95% CI: 1.86–26.02) and 4th quartile: OR 6.67 (95% CI: 1.74–25.67)	–	[34]
		First birth		Higher odds for post-menopausal BC in 4th quartile: OR 6.23 (95% CI: 1.70–22.82) compared to 1st quartile		
		10 years before enrollment		No association with post-menopausal BC: OR 0.85 (95% CI: 0.58–1.22)		
		20 years before enrollment		No association with post-menopausal BC: OR 0.87 (95% CI: 0.61–1.23)		
	Experimental B[a]P	–	4 human breast cancer cell lines	–	No changes in global methylation in all 4 cell lines Decreased methylation of CYP8 and TSC2 in MCF7 and HCC1806	[65]

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NO_2 and NOx	GIS	Adulthood	50–64 year old with no history of BC	No association with BC: HR 1.16 (95% CI: 0.89–1.51)	–	[37]
	Land-use regression	10 year before enrollment	50–74 year old with no history of BC	No association of per IQR increase of 3.75 ppb of NO_2 with BC: OR 1.08 (95% CI: 0.92–1.27)	–	[68]
		20 years before enrollment	Middle aged with BC	Associated with post-menopausal BC: HR 1.04 (95% CI: 1.01–1.08)	–	[32]
		Adulthood	35–74 year old whose sisters had BC (Sister study)	Increased ER+/ PR+ BC: RR 1.10 (95% CI: 1.02–1.19), but not ER–/PR– BC: RR 0.92 (95% CI: 0.77–1.09)	–	[45]
		10 years before enrollment	50–75 year old with BC	Borderline associated with post-menopausal BC: OR 1.31 (95% CI: 1.00–1.71)	–	[70]
		21 years before enrollment		No association with post-menopausal BC: OR 1.17 (95% CI: 0.91–1.50)		
		Adulthood	Middle aged with history of BC (EPIC-Italy and EPIC-Dutch cohort)	–	EPIC Italy – Lower methylation of 11 genes including EPHB2 (β: -0.0046, p: 5.85e–8)	[48]
					EPIC Dutch – No altered methylation of BC genes	
		Prenatal	Newborns	–	Associated with lower cord blood methylation in LonP1, HIBADH and SLC25A28	[72]

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and time of woman's first birth and premenopausal BC (OR: 1.27; 95% CI: 0.48–3.32). Moreover, associations were not evident for estimates of traffic exposure at 10 or 20 year before enrollment (10 years prior, OR: 1.58; 95% CI: 0.71–3.54 and 20 years prior, OR: 1.37; 95% CI: 0.64–2.92) [34]. Other proxies for traffic exposure have included residential history in urban areas [38]. Specifically, Binachon *et al.* evaluated the association of an urban (with presumably higher traffic and other particular airborne pollutants) versus rural address both at birth and during adulthood on BC risk in women from the French E3N cohort (aged 38–66 years at recruitment in 1990). Being born in an urban area was associated with increased BC risk before (HR: 1.11; 95% CI: 1.05–1.18) and after (aHR: 1.07; 95% CI: 1.01–1.14) adjustment for known BC risk factors (basal metabolic rate BMI, alcohol intake, smoking, physical activity). In contrast, living in an urban environment during adulthood was not associated with greater BC risk, implicating the prenatal, but not adult, WOS [38].

Epigenetic findings

The WEB study measured the association between TRAP exposure and epigenetic differences in the breast tissue [34]. Callahan *et al.* examined the DNA methylation patterns among breast tumor samples from women that were recruited in the WEB study using the same estimates of exposure to TRAP [33]. DNA was extracted from needle biopsies of tumor samples particularly from regions with at least 80% tumor cells. Pyrosequencing assays were performed for nine different candidate genes (*SFN, SCGB3A1, RARB, GSTP1, CDKN2A, CCND2, BRCA1, FHIT, SYK*) that were previously reported to be methylated in BC and which demonstrated biological relevance to carcinogenesis. They demonstrated that higher exposure to traffic emissions during menarche was associated with higher methylation of a breast tumor suppressor gene spleen tyrosine kinase (*SYK*; OR: 2.37; 95% CI: 1.05–5.33) [33]. They also reported that cases with higher measures of traffic emissions at first birth and 10 years prior to enrollment demonstrated lower methylation of a breast tumorigenic gene Cyclin D2 (*CCND2*; OR [first birth]: 0.40; 95% CI: 0.17–0.93; OR [10 years prior to enrollment]: 0.48; 95% CI: 0.26–0.89) [33]. These findings suggest that prenatal exposures and adult exposures 10 years prior to diagnosis, may induce epigenetic programming of a BC gene with deleterious effects later in life.

Particulate matter

Particulate matter (PM) generated by internal combustion engines and through secondary processes is a mixture of extremely fine particles and liquid droplets that disperse into the air. PM is classified based on its diameter. These classifications include PM10 (inhalable particles with diameters $\leq$10 μm), PM2.5 (fine inhalable particles with diameters $\leq$2 μm) and PM10–2.5 which is also referred to as PM coarse. PM-related emissions from traffic and other combustion sources may include metals, PAH and other particulates (e.g., sulfur dioxide, metals) [39]. Observations collected from satellite-derived and ground-based measures suggest that PM2.5 levels have increased 0.55 μg/m^3 per year from 1998 to 2012, largely driven by levels in developing countries [40]. Estimates and direct measures of airborne PM, often derived from traffic, also have been associated with higher BC risk [41,42]. In some studies, PM has been shown to be weakly estrogenic and may induce cell proliferation via activation of the estrogen receptor [43,44], providing a mechanism for carcinogenesis in addition to epigenetic ones.

Examples of epidemiological studies that associated PM exposure with higher risk for BC include the Italian population-based cohort based in Varese province, northern Italy involving BC patients, first diagnosed at the age of 50–69 years. Individual adult PM2.5 exposure over the 3 years preceding diagnosis was estimated using satellite observations and the woman's home address. The risk of BC death was higher for all three upper quartiles compared with the lowest in multivariate analyses (HRs of death: second quartile [PM2.5 level 21.10–24.20 μg/m^3]: 1.82 [95% CI: 1.15–2.89], third quartile [PM2.5 level 24.20–26.50 μg/m^3]: 1.73 [95% CI: 1.12–2.67] and fourth quartile [PM2.5 level >26.50 μg/m^3]: 1.72 [95% CI: 1.08–2.75] compared with the first quartile [PM2.5 level <21.10 μg/m^3]) [42]. Bonner *et al.* found, using the WEB study, that higher exposure to TSP (>140 μg/m^3; total suspended particles or particles $\leq$50 μm) at birth, but not at menarche, based on weighted distance of home address to measured TSP concentrations, was not clearly associated with higher adjusted odds of postmenopausal BC (OR: 2.42; 95% CI: 0.97–6.09) compared with low TSP (<84 μg/m^3 at birth) [41]. Other studies, that either focused on chronic PM2.5 and PM10 exposure, such as the US-based Sister Study [45] featuring women with family history of breast cancer, or a shorter time span of 3-year mean levels of PM2.5 and PM10 levels prior to diagnosis, such as the Danish Nurses cohort [46], or of 2-year modeled moving or cumulative average exposures [47], were unable to find associations between PM exposure and BC risk.

Epigenetic findings

There are no data showing direct evidence for PM exposure and increased BC risk mediated via epigenetic regulation. Epigenomic research led by Plusquin *et al.* using the EPIC-Italian and EPIC-Dutch cohorts studied long term (over 10 years after enrollment) levels of PM2.5 and PM10 using a land-use regression model in middle aged women and only found very limited evidence of epigenetic alterations in BC genes. They performed Illumina Infinium® HumanMethylation450 Bead Chip Array on peripheral blood mononuclear cells (PBMCs) from 454 Italian and 159 Dutch participants. Combined analyses involving both cohorts were essentially negative. Upon evaluating site-specific methylation differences by study site, they found significantly lower methylation of only one CpG site (cg03513315) on pescadillo ribosomal biogenesis factor 1 (*PES1*) that was associated with PM10 exposure in the EPIC-Italy cohort (β: 0.22; SE: 0.042; p-value: 1.47e-7) [48]. More breast tissue specific research by Li *et al.* found that *PES1* gene is expressed at higher levels in primary breast cancers compared with normal mammary tissues; knockdown of *PES1* slows down the proliferation of BC cells [49]. Another study reported that *PES1* increases the ERα/ERβ ratio and triggers breast tumor growth [50].

Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAH) are the by-products of combustion and enter the human body from a variety of sources, including inhaling gasoline and diesel-fueled engines coal, coke, and oil burners, and eating grilled and smoked meats and smoke from cigarettes [51–53]. PAH are lipophilic, can be stored in the breast fat tissue [54], and have the capacity to bind to the DNA forming PAH–DNA adducts in the breast tissue [57–57]. PAH also have been found to be directly genotoxic and a potent mutagen [58]. The International Agency for Research on Cancer (IARC) has cited PAH as possible human and animal carcinogen [51]. Several studies have shown that in breast epithelial tissues, PAHs are metabolized to their most potent and deleterious state affecting cellular morphology, cellular division, growth and repair [59–61].

Several *in vivo* and epidemiological studies have linked PAH exposure with increased breast tumorigenesis [59–64]. El-Bayoumy *et al.* gavaged 30-day-old female rats once weekly for 8 weeks with a high dose of the PAH benzo[a]pyrene (B[a]P (50 μmol/rat/week in 0.5 ml trioctanoin) and conducted analyses at age 41 weeks. The B[a]P-treated rats developed malignant mammary tumors with increased proliferation and invasion [59]. Two epidemiological studies, the Long Island Breast Cancer Study (LIBSCP) [64] and the aforementioned WEB [34] population-based case–control studies, employed similar traffic-related pollution models using geographic locations that estimated the residential exposure to PAH [34]. The objective in LIBSCP was to evaluate the association between residential exposure to B[a]P, as a proxy for traffic-related PAH and BC incidence. Both recent B[a]P exposure over the preceding 12 months prior to case ascertainment, and long term during 1960–1990 were assessed. Associations were very modest until they stratified women according to tumor hormone receptor subtype. In adjusted models, higher recent B[a]P exposure in the top 5% was associated with ER-/PR- (OR: 2.09; 95% CI: 1.08–4.06), but not ER+/PR+ (OR: 0.74; 95% CI: 0.40–1.38), BC [64]. The WEB study stratified subjects by smoking status and found life-time nonsmokers had higher odds for premenopausal BC associated with PAH measures in the 2nd (OR: 4.89; 95% CI: 1.34–17.83), 3rd (OR: 6.96; 95% CI: 1.86–26.02) and 4th quartiles (OR: 6.67; 95% CI: 1.74–25.67) at menarche [34]. Interestingly, they also observed higher odds for postmenopausal BC (OR: 6.23; 95% CI: 1.70–22.82) among nonsmokers in relation to higher PAH exposure at first birth [34]. PAH can be biotransformed into reactive intermediates that form covalent PAH–DNA adducts. These may cause DNA damage and possess mutagenic properties to initiate and/or promote breast tumorigenesis [52]. LIBSCP also found significantly higher odds of BC for women with detectable levels of PAH–DNA in blood in the highest versus lowest quantile of exposure (OR [highest quantile]: 1.51; 95% CI: 1.04–2.20; vs OR [lowest quantile]: 1.13; 95% CI: 0.71–1.81) [62]. Moreover, the association between recent adult B[a]P exposure in the Long Island Breast Cancer study project (LIBCSP) cohort and BC incidence was more evident among women with variant alleles in the DNA repair genes ERCC2 (OR: 2.09; 95% CI: 1.13–3.90) and XRCC1 (OR: 2.32; 95% CI: 1.22–4.49) [63].

Epigenetic findings

PAH-induced altered DNA methylation has been found in both breast cell lines and breast tissues. For example, MCF7, HCC1806, T47D and MDA-MD-231 BC cell lines were treated with multiple doses of B[a]P, and global and site-specific changes in DNA methylation patterns were characterized repeatedly over 96 h in culture. While B[a]P did not induce changes in global methylation in all four cell lines, numerous site-specific changes in DNA methylation using amplification of intermethylation sites (AIMS) were detected. These included B[a]P-induced

decreases in methylation of *CYP8*, a member of the cytochrome *P*450 superfamily in the MCF7 cells on 96-h treatment, as well as multiple B[a]P-induced decrease in methylation of genomic repeat elements and several short interspersed nucleotide elements (SINEs), including one at the telomeric site of the tumor suppressor gene tuberous sclerosis complex 2 (*TSC2*) in MCF7 and HCC1806 cell lines. While these tissue culture experiments are limited in their ability to predict *in vivo* events, lower methylation in these regions have been associated with losses in genomic stability which has been implicated in carcinogenesis [65].

In the LIBCSP cohort, White *et al.* assessed the association between PAH–DNA adducts in blood and breast tumor tissue stratified by BC candidate gene DNA methylation levels and tumor subtypes. The list of 13 BC candidate genes studies were known to play an important role in breast tumorigenesis and their promoter regions were frequently methylated in breast tumor tissues [31,66]. Women with detectable PAH–DNA adducts and significantly higher methylation of the steroid hormone receptor retinoic acid receptor β (*RARβ*) gene in breast tumor tissue (OR: 2.69; 95% CI: 1.02–7.12) were more likely to have ER+/PR+ tumors compared with other subtypes. Differences in global methylation levels assessed in the blood by PAH–DNA adducts were absent [66]. White *et al.*, in a subsequent LIBCSP study, focused on the impact of multiple sources of PAH exposure, including estimates of traffic-related B[a]P exposure and nontraffic sources, such as current smoking and synthetic log burning, whose mechanisms could mimic those from PAH emitted from traffic-related sources. Report of current smoking was associated with lower breast tissue methylation for death-associated protein kinase 1 (DAPK) a breast tumorigenic gene (OR: 0.53; 95% CI: 0.28–0.99) and higher B[a]P estimates were associated with higher methylation of TWIST, a gene that promotes epithelial–mesenchymal transition (OR: 2.79; 95% CI: 1.24–6.3). Moreover, nontraffic PAH sources from report burning of synthetic logs was associated with higher methylation of *RARβ* (OR: 1.80; 95% CI: 1.16–2.78), tumor suppressor gene hairpin-induced gene 1 (*HIN1*; OR: 2.14; 95% CI: 1.34–3.42) and E-cadherin (*CDH1*) (OR: 2.26; 95% CI: 1.06–4.79) and lower methylation of BRCA1 (OR: 0.44; 95% CI: 0.30–0.66) and *LINE-1* (OR: 0.59; 95% CI: 0.41–0.86) [31].

Nitrogen dioxide (NO_2) & Nitrogen oxide (NOx)

NO_2 emissions are primarily derived from the burning of fuel and vehicle exhaust, including those emitted from cars, trucks and buses, power plants and off-road equipment [32,67], and contribute to the formation of photochemical smog [68]. There is recent evidence of increasing levels of NO_2 and NOx from traffic emission levels in cities [69]. NO_2 levels have been shown to be associated with higher BC incidence in several [45,70] – but not all – studies [37,68]. Goldberg *et al.* conducted a population-based, case–control study of street-level concentrations of NO_2 (10 years prior to recruitment) and incident postmenopausal BC in Montreal, Canada using a land-use regression model [68]. They did not find an increased OR for postmenopausal BC (OR: 1.08; 95% CI: 0.92–1.27) per interquartile range increase of 3.75 p.p.b. of NO_2 [68]. Another study combining 15 cohorts from nine European countries focusing on a large group of postmenopausal women estimated residence and traffic-related NO_2 and NOx pollution exposure using a land-use regression model [32]. They found that higher NOx exposure over the preceding 20 years was associated with higher incidence of postmenopausal BC (HR: 1.04; 95% CI: 1.01–1.08 per increment of 20 $\mu g/m^3$ NOx) [32]. The Sisters Study, as previously mentioned, also estimated adult exposure to NO_2 using a land-use regression model based on the annual average of (NO_2) levels outside their residence using monitoring data since 1990 until the time of diagnosis and its association to BC risk [45]. They found that each interquartile range difference of 5.8 p.p.b. NO_2 exposure was associated with increased risk of ER+/ PR+ BC (n = 947 ER+/PR+ cases; RR: 1.10; 95% CI: 1.02–1.19), but not ER-/PR- BC (n = 223 ER-/PR- cases, RR: 0.92; 95% CI: 0.77–1.09) [45]. In a Canadian-based case–control study, postmenopausal women aged 50–76 years were recruited. Recent NO_2 levels were measured at time of recruitment using air samplers located at 22 locations and land-use regression models and distant NO_2 exposure 10 and 21 years previously was estimated based on home addresses. For each increase in 5 p.p.b. NO_2 estimated during the previous 10-year period, the adjusted OR for postmenopausal BC was 1.31 (95% CI: 1.00–1.71). No association with postmenopausal BC and NO_2 exposure 21 years prior (OR: 1.17; 95% CI: 0.91–1.50) [70]. Otherwise, to our knowledge, there are no NO_2 exposure studies that focused on younger WOS and BC risk assessment.

Epigenetic findings

There are no data showing direct evidence for NO_2/NOx exposure and increased BC risk mediated via epigenetic regulation. However, Plusquin *et al.* used an epigenome-wide DNA methylation approach in the EPIC cohort to study the link between exposure to NO_2 assessed by using a land-use regression model and DNA methylation in

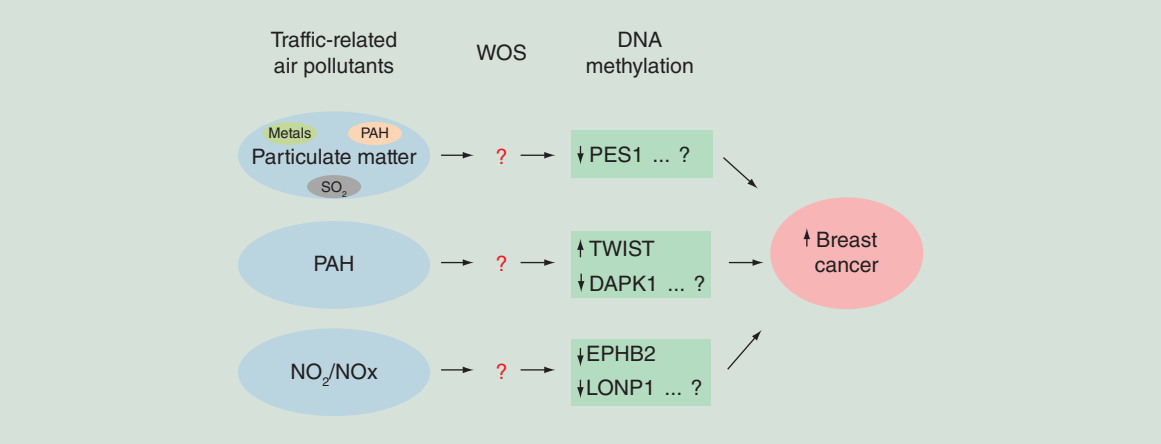

Figure 1. Traffic-related air pollutants, windows of susceptibility, evidence of epigenetic regulation and breast cancer risk. Multiple traffic-related emissions, during several WOS that still need to be determined, have been associated with DNA methylation of genes implicated in breast cancer risk. As examples, traffic-related particulate matter may hypomethylate pro-tumorigenic gene *PES1*; exposure to PAH from non-traffic sources has been associated with hypermethylation of the epithelial mesenchymal transition factor TWIST and hypomethylation a protumorigenic gene DAPK1; NO_2 exposure may hypomethylate protumorigenic genes EPHB2 and LONP1, explaining its association with higher breast cancer risk. Apart from epigenetic processes, the contributions of other pathways (eg. DNA damage) still need to be elucidated.
DAPK1: Death associated protein kinase 1; EPHB2: Ephrin type-B receptor 2; LONP1: Lon protease 1; PAH: Polycyclic aromatic hydrocarbon; PES1: Pescadillo Ribosomal Biogenesis Factor 1; WOS: Windows of susceptibility.

peripheral blood mononuclear cells [48]. They found NO2 exposure was associated with lower methylation of 12 different CpG sites in 11 different genes, including breast tumor suppressor gene Ephrin type-B receptor 2 (*EPHB2*) in the EPIC-Italy only [48]. EPHB2 belongs to the family of Eph receptor tyrosine kinase receptors and has been to shown to be overexpressed in BC as well as to play a role in BC development [71]. Gruzieva *et al.* meta-analyzed the associations between prenatal NO_2 exposure as indicated by residential addresses at birth using a land-use model and cord blood DNA methylation in four European and North American studies (n = 1508). Prenatal NO_2 measures were associated with lower offspring DNA methylation in cord blood of mitochondria-related genes (CpG sites: cg12283362, gene: *LonP1*; cg24172570, gene: *HIBADH* and cg08973675, gene: *SLC25A28*) [72]. Interestingly, *LonP1* is essential in BC development and growth [73], suggesting a possible link between prenatal NO_2 exposure and increased BC risk via lower methylation and thereby activation of *LonP1*.

Conclusion

Does exposure to TRAP associate with the development of breast cancer?

As summarized in Table 1, the evidence favors this conclusion. Associations between TRAP components PAH and NO_2 and BC risk were more consistent. Nie *et al.* have examined exposures specifically during menarche and the woman's first birth [34]. However, the time WOS to exposure appear less specific in many other studies. Most assessed adult exposure to TRAP exposure over a broad period of time, often measured or estimated in the decades before diagnosis, neglecting possibly other key time windows during puberty, pregnancy and lactation when the breast tissue undergoes major structural changes. Further, a widespread bias against publishing negative results may impact our ability to understand fully the impact of all recent research.

Is BC a result of epigenetic responses to TRAP?

As described above, the supporting data directly implicating epigenetic alterations are limited (Figure 1). However, we still suspect these mechanisms are important. Mounting evidence also supports the importance of epigenetic regulation to breast carcinogenesis, even when environmental sources are not examined. One example is the work from Lessons in Epidemiology and Genetics of Adult Cancer from Youth (LEGACY) girls cohort study that recently found differentially methylated regions (DMRs) associated with BC family history [74]. LEGACY investigators studied DNA methylation patterns in white blood cells for 29 candidate BC genes selected from previous publications in 426 girls (ages 6–13 years), in which 239 were reported with, and 187 without, a BC

family history. Using targeted bisulfite sequencing they found differentially methylated regions in two genes, estrogen receptor 1 (*ESR1*) and SEC16 homolog B, endoplasmic reticulum export factor (*SEC16B*). Both of these genes have been reported to play a key role in BC susceptibility and pubertal development, suggesting that their differential methylation may indicate risk early and prior to adult onset disease [74]. In a nested case–control study within the prospective Melbourne Collaborative Cohort Study, Severi *et al.* tested the association between epigenome-wide DNA methylation levels using Illumina Infinium Human Methylation 450 Bead Chip array in peripheral blood with the odds of diagnosis of breast cancer. The odds of later developing BC was compared with mean methylation levels of various regions in the epigenome, including relative to CpGs islands; relative to repetitive elements and in the gene promoter [75]. Higher mean levels of DNA methylation of CpGs located within all functional promoter regions were associated with a higher odds of BC (OR: 1.59; 95% CI: 1.07–2.36); whereas, higher DNA methylation of genomic regions outside promoters was associated with lower odds of BC (OR: 0.69; 95% CI: 0.52–0.93) [75]. These results suggest that epigenomic analyses, even in in peripheral blood, collected before diagnosis may have utility as predictive biomarkers and thus for early detection of breast cancer.

Future perspective

Given the emerging importance of environmental epigenetic regulation in the development of a number of complex diseases [24,29,30,76,77], and the growing literature on TRAP and BC risk, more research is needed to elucidate mechanisms and demonstrate causal effects. Epidemiological studies should focus on discrete WOS, individually or in combination, when exposure to traffic-related air pollutants may exert its largest effects on BC risk. They need to consider potential confounders like diet, obesity and access to BC screening [41,64]. Also, sufficiently powered statistical tests for mediation are needed to inform more mechanistically on the importance of epigenetic regulation to explaining the links between TRAP exposure and cancer. Translational research should be paired with determination of epigenomic biomarkers. Controlled models that experimentally expose animals to traffic-related air pollutants may also lead to a greater understanding of the tissue-specific signaling pathways, including potential epigenetic mechanisms, that underlie BC risk. In addition, much needed experimental studies may inform further alternate pathways besides epigenetic regulation, such as PM-induced endocrine disruption [43,44] and PAH-induced DNA damage [52], that could underlie BC risk.

The gaps in our understanding of epigenetic regulation of BC risk following exposure to air pollution compared with that in association with other tissues and diseases are striking. For example, Hesselbach *et al.* analyzed the impact of ambient PM2.5 from biomass combustion on the transcriptome and methylome of primary human bronchial epithelial BEAS-2B cells using the Affymetrix HG-U133 Plus 2.0 Array and Illumina HumanMethylation450 Bead Chip, respectively. They found PM2.5-induced differentially methylated CpGs in the CpG islands, including differentially methylated CpGs in the promoter region. These PM2.5 induced differentially methylated genes correlated with their mRNA expression and were functionally linked to pathways that play a prominent role in lung cancer [24]. Another study investigated the association of prenatal PM2.5 and PM10 exposure estimates from Southern California Children's Health Study air quality monitoring data based on residential address at birth on DNA methylation (Human Methylation 450 Bead Chip) of 178,309 promoter regions in archived bloodspots from 240 newborns. Breton *et al.* reported an association between prenatal PM2.5 and PM10 exposure at birth and higher methylation in the promoter region of transmembrane 9 superfamily member 2 (*TM9SF2*), ubiquitin-conjugating enzyme E2 (*UBE2*S) and lower methylation of tudor domain containing 6 (*TDRD6*) genes which may be associated with asthma [76]. Gref *et al.* sought to determine gene–environment interaction effects on childhood asthma in three European cohorts using genome-wide single-nucleotide polymorphism (SNP) data from Affymetrix Genome169 wide Human SNP array 5.0 and outdoor NO_2 exposure using a land-use regression model. Several SNPs showed evidence of positive interactions between NO_2 exposure and childhood asthma. Lower methylation was found in one of them, the differential discs, large homolog 2 (*DLP2*) gene. Lower methylation of *DLP2* was also replicated in blood samples obtained during *in vivo* experiments prior to and following 2 h of diesel exposure (300 μg/m^3, containing high levels of NO_2 at 0.22 ppm) within a small group of nonsmoking asthmatics [77].

In summary, future research studies that conduct epigenome-wide studies, with attention to tissue specificity, following exposure to TRAP across key WOS on increased BC risk, are greatly needed. We expect these to occur in the upcoming years. Hopefully such research will identify environmental exposures of concern, allowing for interventions and greater surveillance for those at high risk for onset of future disease.

Executive summary

Traffic-related air pollutant exposure & breast cancer risk

- Emerging data suggest that higher concentrations of traffic pollutants are associated with elevated breast cancer (BC) risk.
- Breast tissue may be susceptible to environmental toxicants during prenatal, pubertal and pregnancy time periods of exposure due to its changing form and function.
- Environmental toxicants may exert effects through alteration of epigenetic regulatory mechanisms.

Vehicular traffic measures

- The association between vehicular traffic measures and BC risk appears inconsistent.
- Higher exposure to traffic exposure during menarche was associated with higher odds of premenopausal breast cancer and higher methylation of breast tumor suppressor gene spleen tyrosine kinase (*SYK*).

Particulate matter

- The association between traffic-related particulate matter (PM) exposure and BC risk appears inconsistent.
- PM exposure during adulthood may lower methylation of breast protumorigenic gene *PES1*.

Polycyclic aromatic hydrocarbons

- Traffic-related polycyclic aromatic hydrocarbons (PAH) exposure appears associated with increased BC risk.
- Women with detectable PAH–DNA adducts and methylated steroid hormone receptor *RARβ* were more likely to have ER+/PR+ tumors.

Nitrogen dioxide (NO_2) & nitrogen oxide (NOx)

- Traffic-related NO_2 exposure is associated with increased BC risk.
- Traffic-related NO_2 may lower methylation of protumorigenic genes *EPHB2* and *LONP1*.

Future perspective

- Epigenome-wide research across possible time windows of susceptibility in mammary tissue and on BC risk is greatly needed.
- These should pay particular attention to tissue specificity following exposures to traffic-related air pollution.

Author contributions

The authors D Sahay, MB Terry and R Miller co-wrote and edited this article.

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