

RESEARCH

Open Access



Genotoxic and epigenotoxic effects in mice exposed to concentrated ambient fine particulate matter (PM_{2.5}) from São Paulo city, Brazil

Antonio Anax Falcão de Oliveira^{1†}, Tiago Franco de Oliveira^{1,5†}, Michelle Francini Dias¹, Marisa Helena Gennari Medeiros², Paolo Di Mascio², Mariana Veras³, Miriam Lemos³, Tania Marcourakis¹, Paulo Hilário Nascimento Saldiva^{3,4} and Ana Paula Melo Loureiro^{1*} 

Abstract

Background: The Metropolitan Area of São Paulo has a unique composition of atmospheric pollutants, and positive correlations between exposure and the risk of diseases and mortality have been observed. Here we assessed the effects of ambient fine particulate matter (PM_{2.5}) on genotoxic and global DNA methylation and hydroxymethylation changes, as well as the activities of antioxidant enzymes, in tissues of AJ mice exposed whole body to ambient air enriched in PM_{2.5}, which was concentrated in a chamber near an avenue of intense traffic in São Paulo City, Brazil.

Results: Mice exposed to concentrated ambient PM_{2.5} (1 h daily, 3 months) were compared to in situ ambient air exposed mice as the study control. The concentrated PM_{2.5} exposed group presented increased levels of the oxidized nucleoside 8-oxo-7,8-dihydro-2'-deoxyguanosine in lung and kidney DNA and increased levels of the etheno adducts 1,N⁶-etheno-2'-deoxyadenosine and 1,N²-etheno-2'-deoxyguanosine in kidney and liver DNA, respectively. Apart from the genotoxic effects, the exposure to PM_{2.5} led to decreased levels of the epigenetic mark 5-hydroxymethylcytosine (5-hmC) in lung and liver DNA. Changes in lung, liver, and erythrocyte antioxidant enzyme activities were also observed. Decreased glutathione reductase and increased superoxide dismutase (SOD) activities were observed in the lungs, while the liver presented increased glutathione S-transferase and decreased SOD activities. An increase in SOD activity was also observed in erythrocytes. These changes are consistent with the induction of local and systemic oxidative stress.

Conclusions: Mice exposed daily to PM_{2.5} at a concentration that mimics 24-h exposure to the mean concentration found in ambient air presented, after 3 months, increased levels of DNA lesions related to the occurrence of oxidative stress in the lungs, liver, and kidney, in parallel to decreased global levels of 5-hmC in lung and liver DNA. Genetic and epigenetic alterations induced by pollutants may affect the genes committed to cell cycle control, apoptosis, and cell differentiation, increasing the chance of cancer development, which merits further investigation.

Keywords: Particulate matter, Oxidative stress, DNA adducts, DNA methylation

* Correspondence: apmlou@usp.br

[†]Antonio Anax Falcão de Oliveira and Tiago Franco de Oliveira contributed equally to this work.

¹Departamento de Análises Clínicas e Toxicológicas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Av. Prof. Lineu Prestes 580, Bloco 13 B, São Paulo CEP 05508-000, Brazil

Full list of author information is available at the end of the article



Background

Epidemiologic studies conducted mostly in the United States and Europe have shown that cardiovascular diseases, respiratory diseases, and cancer of different tissues (e.g., lung, breast, skin, hematopoietic, bladder, kidney, larynx, and thyroid) are empirically associated with exposure to air pollution [1–12]. Nonetheless, the balance between polluting activities and public policies to control air pollution dictates specific air pollutant compositions in each region, whose effects on disease risk, particularly cancer, are far from being completely understood [9].

The atmospheric chemical composition of the Metropolitan Area of São Paulo is characterized by emissions of approximately 2000 industries with high pollution potential and a fleet of approximately 7 million vehicles, which are the main sources of air pollutants in a region inhabiting approximately 21 million individuals [12, 13]. Automotive fuels in use are gasohol (gasoline containing 20–25% ethanol), hydrated ethanol (95% ethanol), compressed natural gas, and diesel containing 5% biodiesel [12], creating a unique composition of atmospheric pollutants with increased levels of formaldehyde, acetaldehyde, and ethanol compared to those found in other countries [14, 15]. Policies to reduce pollutant emissions by vehicles and industries in São Paulo State have been implemented since the 1980s and a diminishing tendency of regulated air pollutants (CO, hydrocarbons, nitrogen oxides, PM₁₀, and sulfur oxides) has been observed, except for ozone, even with the increase in vehicular fleet and fuel consumption [12]. However, the current levels in São Paulo are far from the recommended limits of the WHO [16], and high positive correlations between air pollution exposure and risk of cardiovascular disease, cancer, and mortality have been observed, as also shown in developed countries [7, 17–19].

The particulate matter (PM) fraction of air pollution includes solid and liquid particles of aerodynamic diameters ranging from 5 nm to 100 µm, originating from fuel combustion, biomass burning, and nucleation events based on vapor condensation (sulfuric acid, nitric acid, organic matter) in the atmosphere [9, 12, 18]. Coarse PM, with a 2.5 to 10 µm aerodynamic diameter (PM₁₀), are retained in the upper airways, while fine and ultrafine PM, with aerodynamic diameters in the 0.1–2.5 µm (PM_{2.5}) range and smaller than 0.1 µm (PM_{0.1}), respectively, can penetrate into the terminal portion of bronchi and alveoli [4, 7, 18], where they can elicit inflammatory reactions that increase the risk of diseases [2, 20, 21]. In addition to the lung absorption of particle compounds, airway mucociliary clearance results in gastrointestinal tract exposure to swallowed particles, which may trigger systemic effects [22]. Fine particles are important carriers of organic and inorganic chemicals, such as polycyclic aromatic hydrocarbons (PAHs), nitro-PAHs, aldehydes, ketones, carboxylic acids, quinolines, metals, and water-soluble ions, which may lead

to harmful effects, such as inflammation, oxidative stress, mutations and cancer [10, 23–25]. Carcinogenic PAHs, such as benzo[*a*]pyrene, can be metabolized to reactive intermediates able to damage biomolecules, including the DNA [26, 27]. Diverse types of genome damage (chromosomal aberrations, DNA strand breaks, sister chromatid exchange, DNA adducts, micronuclei) and mutations have been shown as consequences of exposure to benzo[*a*]pyrene [26, 27]. The increase in the generation of reactive oxygen species (ROS) induced by PAHs may also contribute to the observed effects [28]. Based on strong evidence from epidemiological and experimental studies, the International Agency for Research on Cancer (IARC), in 2013, classified outdoor air pollution and airborne particulate matter as carcinogenic to humans (Group 1) [10].

Among the useful biomarkers for the assessment of chemical carcinogenesis pathways, DNA adducts and oxidized nucleobases or nucleosides provide information on early events triggered by exposure, such as the bioactivation of xenobiotics and oxidative stress, while DNA methylation changes depict early biological effects that may evolve to cancer [5]. Accordingly, the levels of the oxidized base 8-oxo-7,8-dihydro-guanine (8-oxoGua) or the deoxyribonucleoside 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo, Fig. 1) are consistently increased in the mononuclear blood cells and urine of subjects exposed to ambient air pollution [5]. Regarding cancer risk, lung 8-oxodGuo levels were correlated with the induction of lung tumors in mice exposed to diesel exhaust particles (DEPs) [29], while in humans the urinary excretion of 8-oxodGuo was associated with the risk of lung cancer [30] and the incidence of colorectal cancer and benign adenoma [31].

In addition to direct DNA oxidation, oxidative stress may induce the formation of mutagenic exocyclic DNA adducts, such as propano, etheno, and malonaldehyde adducts, via reactive carbonyls resulting from lipid peroxidation [32]. The potential role of these lesions as biomarkers of oxidative stress and in cancer etiology has been determined since their quantification in vivo became feasible by using highly sensitive and selective methods [33–43]. Interestingly, the etheno adducts 1,*N*²-etheno-2'-deoxyguanosine (1,*N*²-edGuo, Fig. 1) and 1,*N*⁶-etheno-2'-deoxyadenosine (1,*N*⁶-edAdo, Fig. 1) were the most increased (3- to 4-fold) DNA lesions among others investigated (DNA oxidation and deamination products) in spleen, liver and kidney DNA samples from the nitric oxide overproduction SJL mouse model of inflammation [37]. Despite their potential use as biomarkers in the pathophysiology of inflammation [36, 37], the assessment of these etheno adducts under conditions of PM exposure has only been addressed by a few studies focusing on the effects of wood smoke and ambient PM resulting from wood stoves [44, 45]. Regardless of the inflammatory response [20, 21, 46–48], there are no studies on the effects of urban air PM exposure on the tissue levels of DNA etheno adducts.

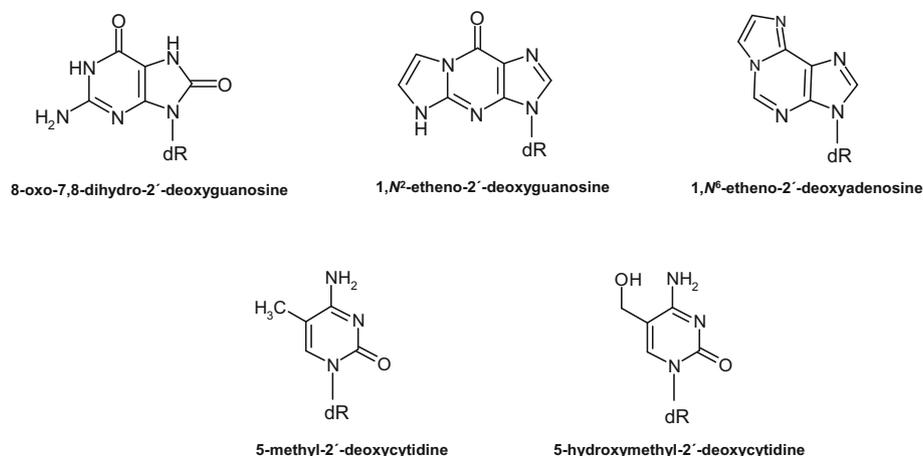


Fig. 1 Chemical structures of the DNA lesions and epigenetic marks quantified in the present study. dR = 2'-deoxyribose

Current research has uncovered epigenetic changes associated with PM exposure, which may play a role in cardiovascular disease and cancer development [47, 49–52]. The current best characterized epigenetic mark is DNA 5-methylcytosine (5-mC, Fig. 1), maintained by DNA methyltransferases (DNMTs) at approximately 4% of the cytosine levels in adult human DNA, with important roles in development, genomic imprinting, silencing of transposable elements, and regulation of gene expression [50, 53–56]. While DNMTs catalyze the C-5 methylation of cytosine bases pertaining to CpGs sequences, the Ten-Eleven-Translocation (TET) methylcytosine hydroxylases are involved in the oxidation of 5-mC to 5-hydroxymethylcytosine (5-hmC, Fig. 1), which is considered the first step in the demethylation process [57].

Studies on global DNA methylation in the whole blood of humans have been associated with a hypomethylation effect of PM exposure [47, 49, 51, 58–62], whereas the outcomes of PM exposure on the global DNA methylation of other target tissues have been studied in mice sperm, rat lung, human placenta, and human buccal cells [63–67]. Increased levels of 5-hmC were detected in blood samples from humans exposed to ambient PM₁₀ [68], but human bronchial epithelial cells (HBECs) exposed to DEPs (5 mg/cm², 24 h) *in vitro* presented decreased 5-hmC levels [69]. Additionally, the increase in human exposure to PM_{2.5} or PM₁₀ was associated with a decrease of 5-hmC levels in buccal cells [65].

To better understand the toxicity pathways of fine particulate matter, considering that studies focusing on the genotoxic and epigenotoxic effects of ambient air pollution in South America are scarce [5, 8], we used HPLC-ESI-MS/MS methods to evaluate the effects of ambient PM_{2.5} on genotoxic (8-oxodGuo, 1,N²-edGuo and 1,N⁶-edAdo lesions) and global DNA 5-mC and 5-hmC changes in different tissues (lung, liver and kidney) of AJ mice. The

animals were exposed whole body to ambient air enriched in PM_{2.5} and compared to *in situ* ambient air exposed mice as the study control. The fine particulate matter was concentrated in a chamber placed near an avenue of intense traffic in São Paulo City, Brazil.

Methods

Chemicals and enzymes

All the chemicals employed here were of the highest purity grade commercially available. Chromatography grade acetonitrile and methanol, isopropyl alcohol, chloroform, hydrochloric acid, ethanol, magnesium chloride, and tris(hydroxymethyl)-aminomethane were obtained from Carlo Erba Reagents (Milan, Italy). Sodium hydroxide, potassium phosphate, and ammonium acetate were acquired from Merck (Darmstadt, Germany). DNA extraction solutions were obtained from QIAGEN (Valencia, CA). DNase I was acquired from Bio Basic Inc. (Ontario, Canada). [¹⁵N₅]-2'-deoxyguanosine and [¹⁵N₅]-2'-deoxyadenosine were provided by Cambridge Isotope Laboratories (Andover, MA). All the other reagents were obtained from Sigma-Aldrich Co. (St. Louis, MO). The catalogue numbers of the reagents are described in Additional file 1: Table S1. Water was purified in a Milli-Q system (Millipore, Bedford, MA).

Adduct standards

1,N²-Etheno-2'-deoxyguanosine (1,N²-edGuo) and the isotopic standards [¹⁵N₅]-1,N²-edGuo, [¹⁵N₅]-1,N⁶-edAdo and [¹⁵N₅]-8-oxodGuo were prepared as described [35, 70]. Their identities were confirmed by their spectral properties, as follows. 1,N²-edGuo: UV, λ_{max} = 285 nm, ε = 16,785 M⁻¹ cm⁻¹ [71], pH 7.0; positive ESI-MS: *m/z* 292 [M + H]⁺, *m/z* 176 [M - 2'-deoxyribose + H]⁺. [¹⁵N₅]-1,N²-edGuo: positive ESI-MS: *m/z* 297 [M + H]⁺, *m/z* 181 [M - 2'-deoxyribose + H]⁺. [¹⁵N₅]-1,N⁶-edAdo: UV, λ_{max} = 260 nm,

$\epsilon = 10,300 \text{ M}^{-1} \text{ cm}^{-1}$ [72], pH 7.0; positive ESI-MS: m/z 281 $[\text{M} + \text{H}]^+$, m/z 165 $[\text{M} - 2'\text{-deoxyribose} + \text{H}]^+$. [$^{15}\text{N}_5$]8-oxodGuo: UV, $\lambda_{\text{max}} = 294 \text{ nm}$, $\epsilon = 9700 \text{ M}^{-1} \text{ cm}^{-1}$ [73] in water; positive ESI-MS: m/z 289 $[\text{M} + \text{H}]^+$, m/z 173 $[\text{M} - 2'\text{-deoxyribose} + \text{H}]^+$.

5-mC standard

5-methyl-2'-deoxycytidine (5-mC) standard was obtained by HPLC purification from a solution of commercial dCyd (1 mg/mL). Its spectral properties are as follows: UV in H_2O , $\lambda_{\text{max}} 277 \text{ nm}$, $\epsilon = 8500 \text{ M}^{-1} \text{ cm}^{-1}$ [74]; positive ESI-MS: m/z 242 $[\text{M} + \text{H}]^+$, m/z 126 $[\text{M} - 2'\text{-deoxyribose} + \text{H}]^+$. The standard was used for quantification of 5-mC and 5-hmC, but the retention time of 5-hmC was defined using a sample of calf thymus DNA presenting a main peak with m/z 258 $[\text{M} + \text{H}]^+ \rightarrow m/z$ 142 $[\text{M} - 2'\text{-deoxyribose} + \text{H}]^+$.

Experimental groups

Four week old male AJ mice, specific pathogen free, were obtained from the Breeding Center of Laboratory Animals of Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil, and were treated accordingly to the Ethics Committee of the Faculty of Medicine, University of São Paulo (protocol n 1310/09). The animals were submitted to a controlled exposure system in a Harvard Ambient Fine-Particle Concentrator designed to concentrate $\text{PM}_{2.5}$ up to 30 times. It works separating particles from gases by particle inertia using virtual impaction [75]. It has two chambers, one receiving the air concentrated with particles and the other receiving the ambient air. Two experimental groups were considered, eight animals each: one group breathed concentrated $\text{PM}_{2.5}$ and the other group breathed ambient air. They were exposed 1 h/day in the afternoon, 5 days/week, along 3 months (September to November). Between exposures, animals were maintained in plastic cages, at a controlled temperature, in a 12-h light-dark cycle; they also received clean air (HEPA air), food (CR-1 Nuvilab, Colombo, PR, Brazil) and water ad libitum. Immediately after the last exposure, they were anesthetized by i.p. injection of xylazine and ketamine (87.5 mg/kg ketamine and 12.5 mg/kg xylazine). Blood samples were collected, centrifuged (2000 g), and the red blood cells separated for antioxidant enzyme activity assessment. Animals were euthanized by exsanguination. Lung, liver, and kidney were immediately collected, frozen in liquid nitrogen and kept under -80°C until processing.

Site of exposure

The exposure site was the same described by Akinaga and colleagues [76], located in an area of the Medical School of University of São Paulo, 20 m from the roadside and 150 m from a busy traffic crossroad. The Cerqueira César monitoring station of the São Paulo State Environmental Agency (CETESB, São Paulo, Brazil) is

located 160 m from the exposure site. The region is impacted mainly by the emissions of light duty gasohol / hydrated ethanol and heavy duty diesel vehicles.

Exposure chambers

Animals were maintained in special cages that allowed homogeneous distribution of air. Both experimental groups were exposed at the same time and conditions of temperature and pressure. Animals in the 30 times concentrator chamber were exposed daily to $\text{PM}_{2.5}$ (5 days/week, 3 months), constantly monitored by a nephelometer. The calculated $\text{PM}_{2.5}$ concentration (mean \pm SD) for the entire period of exposure was $682 \pm 532 \mu\text{g}/\text{m}^3$. Twice a week, samples of the $\text{PM}_{2.5}$ were collected onto polycarbonate filters for elemental analysis [77]. For determination of specific polycyclic aromatic hydrocarbons in $\text{PM}_{2.5}$, we used a Hi-vol particle sampler to collect these particles on quartz filters. Analyses of these filters were conducted according to Magalhães and coworkers [78].

Antioxidant enzyme activities

Enzymatic activities of glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), and superoxide dismutase (SOD) were determined by spectrophotometric method in lung, liver, kidney, and erythrocytes. Catalase activity (CAT) was evaluated only in erythrocytes. GPx activity was performed using the procedure described by Flohé and Günzler [79]. Tert-butyl hydroperoxide was used as substrate and the formation of oxidized glutathione (GSSG) was indirectly monitored spectrophotometrically through NADPH consumption at 340 nm during 5 min. GR activity was assayed according to Carlberg and Mannervik [80]. The reduction of GSSG to GSH was measured through NADPH consumption and monitored spectrophotometrically at 37°C for 10 min at 340 nm. GST activity assay was conducted measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione, according to Habig [81]. The formation of the complex was monitored spectrophotometrically at 25°C for 5 min at 340 nm. Superoxide dismutase (SOD) activity was measured based on McCord & Fridovich, and Flohé & Ötting methods that use the system hypoxanthine-xanthine as a superoxide anion donor [82, 83]. The $\text{O}_2^{\cdot-}$ production is coupled to the reduction of ferricytochrome C, which is followed spectrophotometrically, allowing for quantitative measurement. SOD in samples converts superoxide free radical to H_2O_2 and O_2 , therefore slowing the rate of ferricytochrome C reduction. During the assay, the absorbance was detected in a spectrophotometer at 550 nm, 25°C . SOD, GPx, GR and GST assays were performed in spectrophotometer Power Wave \times 340 (Bio-Tek Instruments INC, software KC4 v3.0). Catalase activity was evaluated measuring the consumption of hydrogen peroxide (H_2O_2) [84]. The decrease in

absorbance was monitored at 25 °C for 30 s at 240 nm in a spectrophotometer (Biochrom Libra S12). All enzyme activities in lung, liver and kidney are expressed as U/ μ g of protein. The enzyme activities conducted in erythrocytes were corrected by hemoglobin content and expressed as U/g of hemoglobin. Protein and hemoglobin contents were determined by Bradford and Doles[®] reagents, respectively. All enzymatic assays were conducted in triplicate.

DNA extraction

DNA samples from lung, liver, and kidney were extracted using a QIAGEN kit according to manufacturer's instructions for 1 g of animal tissue, maintaining the correct proportions. Briefly, tissue samples (with average weight of 1.0 g) were homogenized with 10 mL of the cell lysis solution (QIAGEN, Cat. No. 158908) plus 0.5 mM deferoxamine. The obtained homogenates were added to 150 μ L of 20 mg/mL proteinase K solution. The samples were homogenized and remained at room temperature overnight. After this period, 40 μ L of ribonuclease A (15 mg/mL) were added and kept 2 h at room temperature. The proteins were precipitated by adding 5 mL of protein precipitation solution (QIAGEN, Cat. No. 158912) and centrifuged at 2000 g for 10 min. The supernatants were transferred to tubes containing 10 mL of cold isopropanol. The precipitated DNA was collected into tubes containing 4 mL of 10 mM Tris buffer, 1 mM deferoxamine, pH 7.0, and extracted three times with 4 mL of a chloroform solution containing 4% of isoamyl alcohol. The DNA was again precipitated by adding 8 mL of absolute ethanol and 0.4 mL of a 5 M NaCl solution, and washed twice with 3 mL of 70% ethanol. After air drying, the samples were suspended in 200 μ L of 0.1 mM deferoxamine solution and stored at -20 °C. The DNA concentration was determined by measuring the absorbance at 260 nm and its purity was established based on the 260/280 nm absorbance ratio.

DNA enzymatic hydrolysis

For analyses of etheno adducts in liver, aliquots containing 150 μ g of DNA were transferred to a final volume of 200 μ L of deionized water. 7.5 μ L of 200 mM Tris/MgCl₂ buffer (pH 7.4), 1.4 μ L of the internal standard solution containing [¹⁵N₅]1,N⁶- ϵ dAdo and [¹⁵N₅]1,N²- ϵ dGuo (250 fmol/ μ L), and 6 μ L (15 units) of deoxyribonuclease I (Bio Basic Inc., Ontario, Canada) were added. The samples were incubated at 37 °C, 90 rpm for 1 h. Then, 6 μ L (0.006 units) of phosphodiesterase I from *Crotalus atrox* (Sigma Aldrich, St. Louis, MO, USA) and 7.5 μ L (15 units) of alkaline phosphatase from bovine intestinal mucosa (Sigma Aldrich, St. Louis, USA) were added, incubating again at 37 °C, 90 rpm for 1 h. Finally, samples were centrifuged at 14,000 g for 10 min. Aliquots of 10 μ L were withdrawn for quantification of deoxynucleosides (dAdo,

dGuo) by HPLC/PDA. The residual volume of sample was submitted to solid phase extraction, as described below.

Analyses of etheno adducts in kidney and lung were performed using 100 μ g of DNA, maintaining the correct proportions of enzymes and the other reagents.

The same procedure was used for quantification of 8-oxodGuo in liver, lung and kidney, using 80 μ g of DNA and 1000 fmol of the internal standard [¹⁵N₅]8-oxodGuo in the injection volume.

Samples containing 12 μ g DNA and 3000 fmol of [¹⁵N₅]1,N⁶- ϵ dAdo were also hydrolyzed for analyses of 5-mC and 5-hmC, using the same procedure adjusted to a final volume of 60 μ L. The hydrolyzed samples were, then, added to 140 μ L of acetonitrile, vortexed for 20 s, centrifuged at 9300 g for 10 min, and aliquots of 20 μ L were injected into the HPLC-ESI-MS/MS system described below.

Solid phase extraction

Samples for the analyses of etheno adducts were pre-purified by solid phase extraction, using SPE-C18 cartridges (30 mg/mL, 33 μ m, 1 mL, Strata-X, Phenomenex, Torrance, CA, Cat. No. 8B-S100-TAK). This step was not performed for quantification of 8-oxodGuo. The cartridges were loaded in the following sequence: 100% methanol, deionized water, hydrolyzed DNA sample, deionized water, 10% methanol, 15% methanol, and 100% methanol. The last elution fraction containing the adducts of interest was collected. Samples were then vacuum dried and resuspended in 83.1 μ L of MiliQ water immediately prior to the HPLC-ESI-MS/MS analysis, to obtain 200 fmol of internal standards in 50 μ L of each sample.

Quantification of DNA lesions, 5-mC and 5-hmC

The levels of 1,N⁶- ϵ dAdo, 1,N²- ϵ dGuo, 8-oxodGuo, 5-mC and 5-hmC in DNA samples were assessed by HPLC-ESI-MS/MS. The analytical system consisted of an Agilent 1200 series HPLC (Wilmington, DE, USA) equipped with a binary pump (Agilent 1200 G1312B), an isocratic pump (Agilent 1200 G1310A), a column oven (Agilent 1200 G1316B), a diode array detector (Agilent 1200 DAD G1315C), and an auto sampler (G1367C Agilent 1200) interfaced with a Linear Quadrupole Ion Trap mass spectrometer, Model 4000 QTRAP (Applied Biosystems/MDS Sciex Instruments, Foster City). The ESI-MS/MS parameters were set in the positive ion mode as described in Additional file 1: Table S2.

Analyses were carried out with multiple reaction monitoring (MRM) by using the following fragmentations: m/z 276 [M + H]⁺ \rightarrow m/z 160 [M - 2'-deoxyribose + H]⁺ and m/z 281 [M + H]⁺ \rightarrow m/z 165 [M - 2'-deoxyribose + H]⁺ for detection of 1,N⁶- ϵ dAdo and respective internal standard [¹⁵N₅]1,N⁶- ϵ dAdo; m/z 292 [M + H]⁺ \rightarrow m/z 176 [M - 2'-deoxyribose + H]⁺ and m/z 297 [M + H]⁺ \rightarrow m/z 181

$[M - 2' \text{-deoxyribose} + H]^+$ for detection of $1,N^2$ - ϵ dGuo and respective internal standard $[^{15}\text{N}_5]1,N^2$ - ϵ dGuo; m/z 284 $[M + H]^+ \rightarrow m/z$ 168 $[M - 2' \text{-deoxyribose} + H]^+$ and m/z 289 $[M + H]^+ \rightarrow m/z$ 173 $[M - 2' \text{-deoxyribose} + H]^+$ for detection of 8-oxodGuo and respective internal standard $[^{15}\text{N}_5]8$ -oxodGuo; m/z 242 $[M + H]^+ \rightarrow m/z$ 126 $[M - 2' \text{-deoxyribose} + H]^+$ for detection of 5-mC; m/z 258 $[M + H]^+ \rightarrow m/z$ 142 $[M - 2' \text{-deoxyribose} + H]^+$ for detection of 5-hmC; m/z 228 $[M + H]^+ \rightarrow m/z$ 112 $[M - 2' \text{-deoxyribose} + H]^+$ for detection of dCyd.

The calibration curves were constructed at the intervals of 367 to 5875 fmol of 8-oxodGuo, with a fixed amount of $[^{15}\text{N}_5]8$ -oxodGuo (1000 fmol); 1 to 40 fmol of $1,N^6$ - ϵ dAdo and $1,N^2$ - ϵ dGuo, with fixed amounts of $[^{15}\text{N}_5]1,N^6$ - ϵ dAdo and $[^{15}\text{N}_5]1,N^2$ - ϵ dGuo (200 fmol); and 150 to 1500 pmol of dCyd, 5 to 500 fmol of 5-mC for quantification of 5-hmC, and 5 to 80 pmol of 5-mC for quantification of 5-mC, with a fixed amount of $[^{15}\text{N}_5]1,N^6$ - ϵ dAdo (300 fmol). Data were acquired and processed using Analyst software 1.4 (Applied Biosystems/MDS Sciex). The molar fractions 8-oxodGuo/dGuo, $1,N^6$ - ϵ dAdo/dAdo, $1,N^2$ - ϵ dGuo/dGuo, 5-mC/(5-mC + 5-hmC + dCyd), 5-hmC/(5-mC + 5-hmC + dCyd) present in each DNA sample were determined. The following chromatography conditions were used for the analyses. All solvents used were previously filtered and degassed.

8-oxodGuo analysis: A 50×2.0 mm i.d., 2.5 μm , Luna C18(2)-HST column (Phenomenex, Torrance, CA) with a C18(2) security guard cartridge, 4.0×3.0 mm i.d. (Phenomenex, Torrance, CA) was eluted with a gradient of 0.1% formic acid (solvent A) and methanol containing 0.1% formic acid (solvent B) at a flow rate of 150 $\mu\text{L}/\text{min}$ and 25 $^\circ\text{C}$, as follows: from 0 to 25 min, 0–15% of solvent B; 25 to 28 min, 15–80% of solvent B; 28 to 31 min, 80% of solvent B; 31 to 33 min, 80–0% of solvent B; 33 to 46 min, 0% of solvent B. The first 16 min of eluent was directed to waste and the 16–32 min fraction was diverted to a second column (150×2.0 mm i.d., 3.0 μm , Luna C18(2)) connected to the ESI source and conditioned by a third isocratic pump with a solution of 15% methanol in water containing 0.1% formic acid (150 $\mu\text{L}/\text{min}$) (Additional file 1: Figure S1). The lesion 8-oxodGuo eluted from the second column at approximately 36 min.

Etheno adducts analyses: A 150×2.0 mm i.d., 3.0 μm , Luna C18(2) column (Phenomenex, Torrance, CA) with a C18(2) security guard cartridge, 4.0×3.0 mm i.d. (Phenomenex, Torrance, CA) was eluted with a gradient of 5 mM ammonium acetate, pH 6.6 (solvent A) and acetonitrile (solvent B) at a flow rate of 130 $\mu\text{L}/\text{min}$ and 25 $^\circ\text{C}$, as follows: from 0 to 10 min, 0% of solvent B; 10 to 39 min, 0–20% of solvent B; 39 to 41 min, 20–75% of solvent B; 41 to 46 min, 75% of solvent B; 46 to 47 min, 75–0% of solvent B; 47 to 60 min, 0% of solvent B. The first 15 min of eluent was directed to waste and the 15–18 min fraction was diverted to the ESI source.

5-mC and 5-hmC analyses: A 150×4.6 mm i.d., 5 μm , Synchronis HILIC(2) column (Thermo Scientific, USA), with a HILIC security guard cartridge, 4.0×3.0 mm i.d. (Thermo Scientific, USA), was eluted with a gradient of acetonitrile (solvent A) and 5 mM ammonium acetate, pH 8.2 (solvent B) at a flow rate of 300 $\mu\text{L}/\text{min}$ and 35 $^\circ\text{C}$, as follows: from 0 to 35 min, 0–40% of solvent B; 35 to 36 min, 40–0% of solvent B; 36 to 56 min, 0% of solvent B. The first 16 min of eluent was directed to waste and the 16–27 min fraction was diverted to the ESI source.

Method validation for 8-oxodGuo quantification in DNA samples

Method accuracy and precision were determined by adding varying amounts of 8-oxodGuo (367, 734, 1469, and 2204 fmol) and a fixed amount of $[^{15}\text{N}_5]8$ -oxodGuo (1000 fmol) to 100 μg of calf thymus DNA and carrying out the enzymatic hydrolysis and analysis. Samples were processed in quadruplicate in two different days. The limit of quantitation (LOQ) was estimated from the lowest amount of 8-oxodGuo injected on column, with S/N = 10.

Method validation for $1,N^6$ - ϵ dAdo and $1,N^2$ - ϵ dGuo quantification in DNA samples

Varying amounts of $1,N^6$ - ϵ dAdo (1, 5, 10, and 20 fmol in the injection volume) and $1,N^2$ - ϵ dGuo (1, 5, 10, and 20 fmol in the injection volume) and fixed amounts of $[^{15}\text{N}_5]1,N^6$ - ϵ dAdo and $[^{15}\text{N}_5]1,N^2$ - ϵ dGuo (200 fmol in the injection volume) were added to 100 μg of calf thymus DNA and the analyses were carried out. Samples were processed in quadruplicate in two different days for method accuracy and precision assessment. Recovery was calculated by adding the internal standards $[^{15}\text{N}_5]1,N^6$ - ϵ dAdo and $[^{15}\text{N}_5]1,N^2$ - ϵ dGuo (200 fmol) to 100 μg of calf thymus DNA before and after solid phase extraction, carrying out the analyses as described above. The DNA used for recovery calculation was contaminated with 7.5 fmol of $1,N^6$ - ϵ dAdo and 20 fmol of $1,N^2$ - ϵ dGuo in the beginning of the process. The limit of detection (LOD) was estimated from the lowest amount of adducts added to the calf thymus DNA sample.

Normal 2'-deoxynucleosides quantification in DNA samples used for analyses of 8-oxodGuo, $1,N^6$ - ϵ dAdo and $1,N^2$ - ϵ dGuo

The quantification of normal 2'-deoxynucleosides was carried out with a Shimadzu (Kyoto, Japan) HPLC system equipped with two LC-20AT pumps, a photo diode array detector (PDA-20AV), an auto-injector (Proeminence SIL-20 AC), and a column oven (CTO-10AS/VP) controlled by a CBM-20A communication module and the software LC-Solution. Elution system was as follows: a 250 mm \times 4.6 mm i.d., 5 μm ,

Luna C18(2) column (Phenomenex, Torrance, CA) attached to a C18(2) guard column (4,0 × 3.0 mm i.d., 4 μm, Phenomenex, Torrance, CA), eluted with a gradient of 0.1% formic acid and CH₃OH (from 0 to 25 min, 0 to 18% CH₃OH; from 25 to 27 min, 18 to 0% CH₃OH; from 27 to 37 min, 0% CH₃OH) at a flow rate of 1 mL/min and 30 °C. The PDA detector was set at 260 nm. Calibration curves were constructed at intervals of 0.05–1 nmol for dGuo and dAdo.

Statistics

Data were expressed as average ± SEM or average ± SD, as indicated in the text and tables. Means between the two groups (ambient air and PM_{2.5}) were compared using t test for 8-oxodGuo, 1,N²-εdGuo, 1,N⁶-εdAdo, 5-mC and antioxidant enzymes activities, or Mann-Whitney test for 5-hmC. Results were considered statistically significant when *P* value was less than 0.05. Normality of the samples was checked by the Kolmogorov-Smirnov test and homogeneity of variances with the Levene test. The statistical analyses were conducted using GraphPad Prism version 6 for Windows (GraphPad Software, San Diego California USA).

Results

Exposure assessment

The elemental characterization of PM_{2.5} is depicted in Table 1. Table 2 presents the mean concentrations of specific polycyclic aromatic hydrocarbons detected in PM_{2.5} collected near the site of exposure.

Antioxidant enzyme activities

Mice exposed to concentrated PM_{2.5} presented changes in lung, liver, and erythrocyte antioxidant enzyme activities, as shown in Table 3. Statistically significant decreased GR and statistically significant increased SOD activities were observed in the lungs, while the liver presented statistically significant increased GST and statistically significant decreased SOD activities. A statistically significant increase in SOD activity was also observed in erythrocytes.

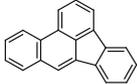
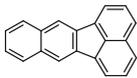
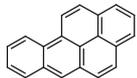
Genotoxic effects of PM_{2.5} in the lungs, liver, and kidneys

The genotoxic effects of PM_{2.5} were assessed by the quantification of 8-oxodGuo and the etheno adducts 1,N²-εdGuo and 1,N⁶-εdAdo in lung, liver, and kidney DNA samples. The levels of each lesion in each organ of mice exposed to PM_{2.5} concentrated 30 times compared to those exposed to ambient air are shown in Table 4.

Table 1 Elements (ng/m³) detected in PM_{2.5} (μg/m³)

	Minimum	Q1	Median	Q3	Maximum	Mean	SD	SEM
PM _{2.5}	65.2	222	574	1047	2094	682	532	95.5
Na	0.00	10.68	432.95	613.42	3774.50	525.64	711.31	125.74
Mg	0.00	0.00	44.13	302.58	6061.46	363.25	1059.70	187.33
Al	289.82	1130.75	1785.09	4219.14	65,846.50	5610.86	11,559.93	2043.53
Si	93.81	827.61	1840.58	4954.59	83,737.34	6489.89	14,685.73	2596.10
P	0.00	39.55	219.26	512.15	8645.44	584.46	1492.15	263.78
S	573.18	2935.17	7074.47	12,747.56	27,164.33	9374.97	8051.74	1423.36
Cl	0.00	75.99	433.59	932.43	4994.57	772.92	1038.12	183.52
K	62.70	778.64	1488.44	3005.62	35,421.57	3458.71	6403.12	1131.92
Ca	44.18	447.99	1233.81	2473.66	40,539.07	3267.85	7148.36	1263.66
Ti	8.70	54.86	151.17	564.01	6261.38	641.99	1429.13	252.64
V	0.00	0.73	6.43	14.18	71.68	11.50	16.50	2.92
Cr	0.00	0.00	0.00	13.09	198.64	14.21	35.27	6.23
Mn	6.21	47.19	90.59	121.04	1432.81	157.48	255.25	45.12
Fe	5.74	1094.30	2042.59	4479.96	65,800.18	5304.88	11,441.17	2022.53
Ni	0.00	0.00	2.96	6.74	81.34	7.99	15.40	2.72
Cu	0.00	23.14	54.24	102.69	868.51	99.66	155.25	27.44
Zn	0.00	146.45	300.87	509.58	3411.48	500.06	703.91	124.43
Se	0.00	0.00	0.00	0.16	10.27	1.24	2.69	0.48
Br	0.00	10.55	15.17	45.03	223.70	36.15	45.36	8.02
Rh	0.00	0.00	0.00	80.53	391.62	51.97	91.40	16.42
Pb	0.00	9.68	31.84	36.13	207.06	37.88	45.23	10.66

Table 2 Polycyclic aromatic hydrocarbons in PM_{2.5} collected at the site of exposure

	Filters (n)	Mean ± SEM (range) ng/m ³
Pyrene 	6	1.66 ± 0.42 (3.6–9.0)
Benz[e]acephenantrylene 	6	6.81 ± 1.88 (2.44–13.97)
Benzo[k]fluoranthene 	8	1.42 ± 0.37 (0.44–3.17)
Benzo[a]pyrene 	7	0.72 ± 0.14 (0.27–1.083)

The levels of 8-oxodGuo were increased in the lung and kidney DNA of mice exposed to concentrated PM_{2.5}, while 1,N²-εdGuo was preferentially formed in the liver DNA of mice exposed to concentrated PM_{2.5}. A significant increase in 1,N⁶-εdAdo was observed in the kidneys of mice exposed to concentrated PM_{2.5}. Representative chromatograms are shown in Fig. 2a and b.

Effects of PM_{2.5} on global 5-mC and 5-hmC in lung, liver, and kidney DNA

Epigenetic effects of PM_{2.5} were evaluated by quantification of 5-mC and 5-hmC in lung, liver, and kidney DNA samples. The levels obtained for each organ from mice exposed to PM_{2.5} concentrated 30 times compared to those of mice exposed to ambient air are shown in Table 4. While exposure to concentrated PM_{2.5} did not lead to statistically significant changes in global 5-mC levels, the lung and liver samples of AJ mice exposed to concentrated PM_{2.5} presented lower levels of 5-hmC. An inverse correlation between 5-hmC and 8-oxodGuo was observed in the lung DNA (Fig. 3a). The ratios 5-mC/5-hmC in the lung DNA also differed significantly between the ambient air and

Table 3 Enzymatic activities of glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) in lung, liver, kidney (U/μg protein), and erythrocytes (U/g hemoglobin)

	Ambient Air Average ± SEM (U/μg protein or U/g hemoglobin)	PM _{2.5} Average ± SEM (U/μg protein or U/g hemoglobin)	Number	P value
Lung				
GPx	2.832 ± 0.083	2.967 ± 0.114	4	NS
GR	1.454 ± 0.047	1.142 ± 0.074	4	0.02
GST	12.96 ± 0.600	12.96 ± 0.480	4	NS
SOD	133,436 ± 5858	190,269 ± 13,951	4	0.02
Liver				
GPx	9.900 ± 0.110	10.11 ± 0.150	5	NS
GR	1.390 ± 0.040	1.350 ± 0.060	5	NS
GST	31.82 ± 1.040	35.25 ± 0.700	5	0.02
SOD	296,609 ± 37,767	142,425 ± 21,046	5	<0.01
Kidney				
GPx	6.540 ± 0.090	6.170 ± 0.230	5	NS
GR	3.110 ± 0.080	2.910 ± 0.140	5	NS
GST	13.37 ± 0.240	13.020 ± 0.570	5	NS
SOD	114,203 ± 7149	109,789 ± 17,576	5	NS
Erythrocytes				
GPx	2175 ± 72.00	2292 ± 63.00	6	NS
GR	103.8 ± 4.500	97.98 ± 3.070	5	NS
GST	168.0 ± 7.900	173.8 ± 3.800	4	NS
SOD	151,304 ± 14,350	216,638 ± 4807	4	<0.01
CAT	110,883 ± 5300	113,442 ± 3196	6	NS

NS Not Significant

Table 4 Levels of DNA lesions, 5-mC and 5-hmC in samples from AJ mice exposed to ambient air and to PM_{2.5} concentrated 30 times

	Ambient Air Average ± SEM	PM _{2.5} Average ± SEM	Number	P value
Lung				
8-oxodGuo/10 ⁸ dGuo	2124 ± 56.96	2466 ± 93.10	6	0.01
1,N ² -εdGuo/10 ⁸ dGuo	ND	ND	–	–
1,N ⁶ -εdAdo/10 ⁸ dAdo	1.41 ± 0.23	1.44 ± 0.13	7	NS
5-mC (%)	3.53 ± 0.18	3.84 ± 0.21	8	NS
5-hmC (%)	0.044 ± 0.006	0.029 ± 0.001	8	<0.01
Liver				
8-oxodGuo/10 ⁸ dGuo	2848 ± 183.5	2949 ± 223.8	6; 5	NS
1,N ² -εdGuo/10 ⁸ dGuo	7.79 ± 2.49	24.94 ± 5.21	4	0.02
1,N ⁶ -εdAdo/10 ⁸ dAdo	2.82 ± 0.30	2.18 ± 0.25	6	NS
5-mC (%)	4.68 ± 0.11	4.36 ± 0.38	8; 6	NS
5-hmC (%)	0.060 ± 0.004	0.051 ± 0.002	8; 6	0.04
Kidney				
8-oxodGuo/10 ⁸ dGuo	1854 ± 87.13	2363 ± 157.0	6	0.02
1,N ² -εdGuo/10 ⁸ dGuo	ND	ND	–	–
1,N ⁶ -εdAdo/10 ⁸ dAdo	1.09 ± 0.15	1.52 ± 0.12	7	0.04
5-mC (%)	4.84 ± 0.24	4.20 ± 0.37	8	NS
5-hmC (%)	0.048 ± 0.003	0.048 ± 0.003	8	NS

NS Not Significant, ND Not Detected

PM_{2.5} groups (Fig. 3b). A representative chromatogram is shown in Fig. 2c.

Method validation for 8-oxodGuo quantification in DNA samples

For 8-oxodGuo quantification, the system illustrated in Additional file 1: Figure S1 was used. A typical calibration curve in the 367–5875 fmol range, with a fixed amount of the internal standard [¹⁵N₅]8-oxodGuo (1000 fmol), is presented in Additional file 1: Figure S2. The MS response was linear in the range measured. The method accuracy and precision are presented in Table 5. Good agreement was observed between added and detected amounts of 8-oxodGuo (86–104% accuracy). The intra-day precision was determined by calculating the coefficient of variation (%) for each concentration. The inter-day precision calculated for DNA aliquots supplemented with 367 fmol of 8-oxodGuo was 16.97%. The LOQ (S/N = 10) was 25 fmol for the standard on-column injection.

Method validation for 1,N⁶-εdAdo and 1,N²-εdGuo quantification in DNA samples

The calibration curves presented in Additional file 1: Figure S2 show that the MS response was linear in the 1–40 fmol range. Good agreement was also observed between added and detected amounts of 1,N⁶-εdAdo and 1,N²-εdGuo (93–101% accuracy for 1,N²-εdGuo, and

100–118% accuracy for 1,N⁶-εdAdo) (Table 5). Intra-day precision was determined by calculating the coefficient of variation (%) for each concentration. The inter-day precision calculated for DNA aliquots supplemented with 10 fmol of 1,N²-εdGuo and 1 fmol of 1,N⁶-εdAdo was 14.01 and 16.66%, respectively. The limits of on-column quantification (S/N = 10) were 0.3 fmol for 1,N⁶-εdAdo and 1 fmol for 1,N²-εdGuo.

Discussion

A complex mixture of gases, vapors and particles of varying size and chemical composition is found in urban air, resultant from diverse polluting human activities [6, 17]. To study the effects of specific components of this complex mixture, taking into account the dynamics of pollutants in real ambient air, a feasible approach is to selectively concentrate the desired component over a background of real pollution [75]. Using this approach, we found here that mice exposed to PM_{2.5} from a busy traffic crossroad in São Paulo City presented altered antioxidant system activity in lung, liver, and erythrocytes, concomitantly to increased levels of lung and kidney 8-oxodGuo, kidney 1,N⁶-εdAdo, and liver 1,N²-εdGuo, as well as decreased levels of lung and liver 5-hmC.

In the present study, mice exposed to ambient air enriched in PM_{2.5} (682 ± 532 μg/m³) for 1 h daily for 3 months (from infancy to adulthood) were compared to a

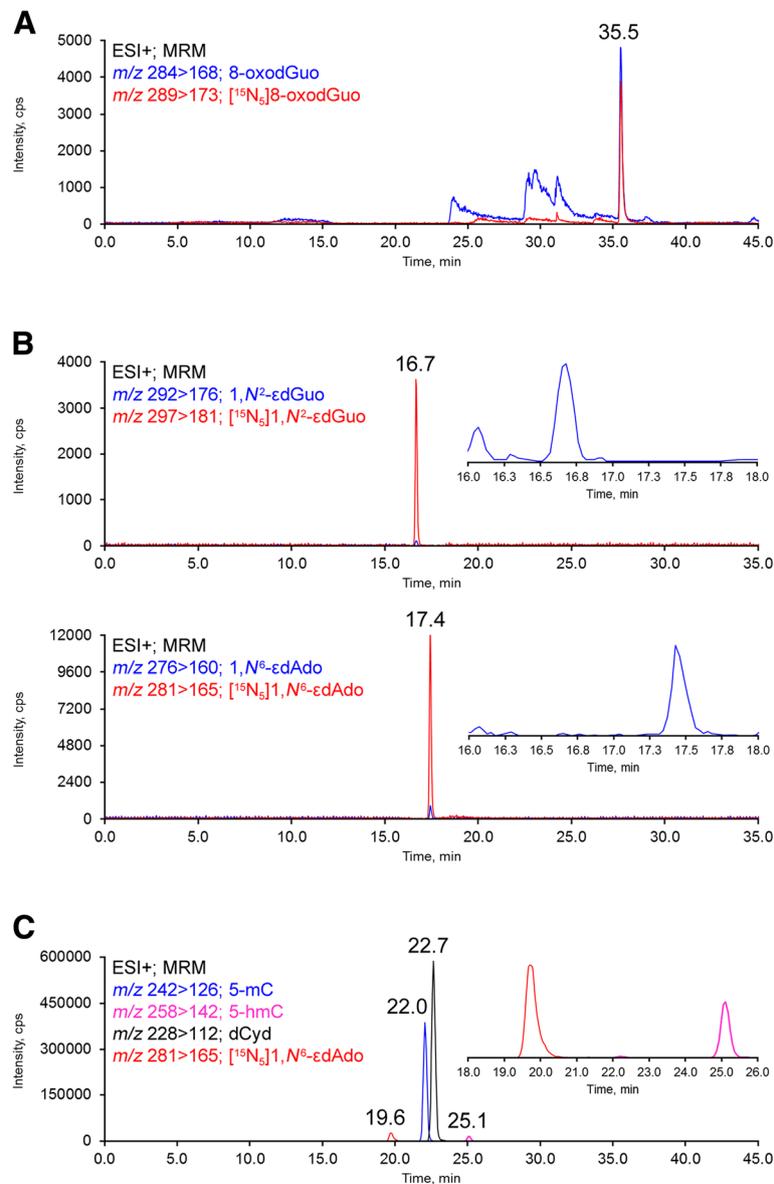
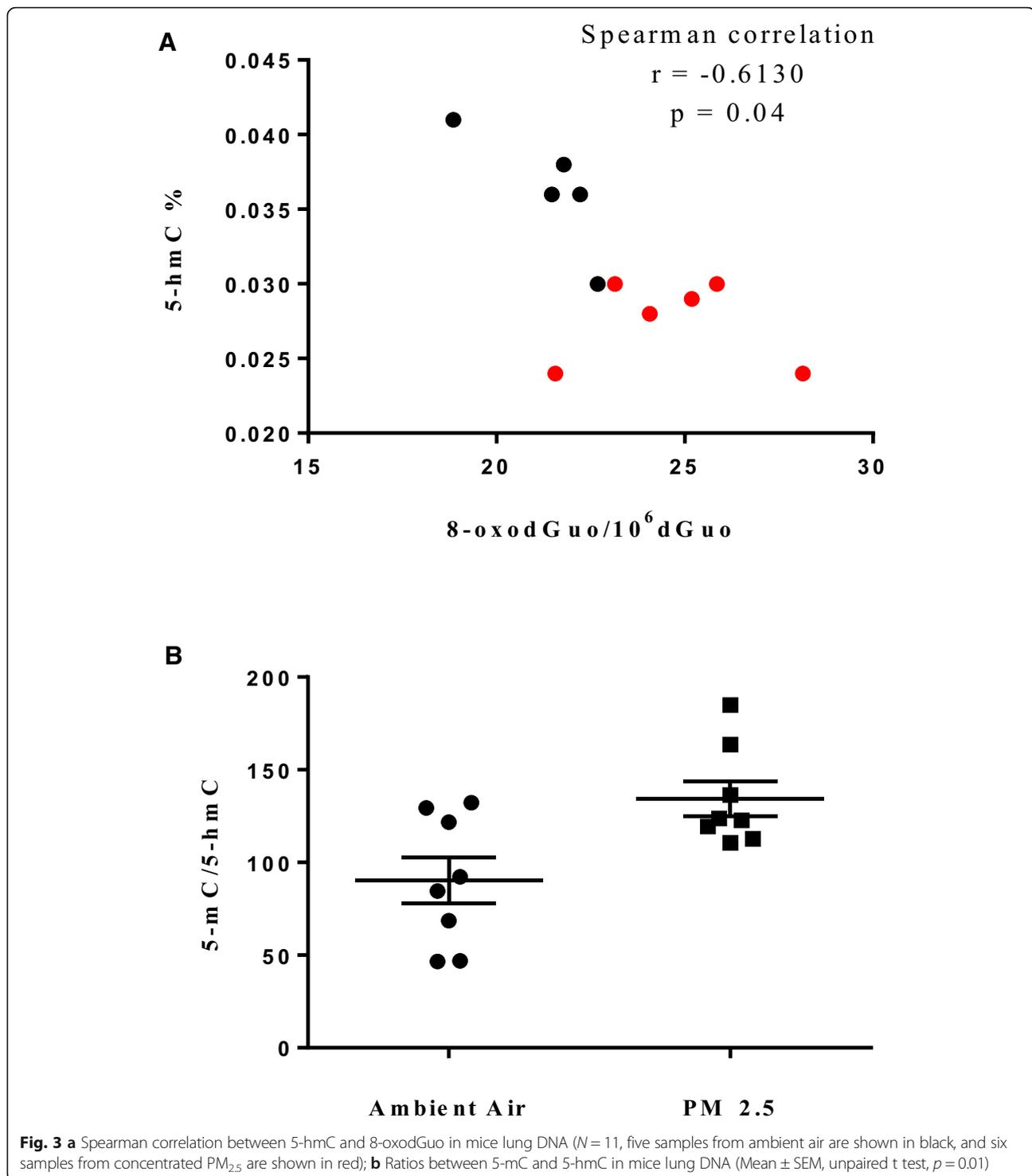


Fig. 2 Representative chromatograms of DNA samples obtained by HPLC-ESI-MS/MS showing **a** 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo); **b** 1,N²-etheno-2'-deoxyguanosine (1,N²-εdGuo) and 1,N⁶-etheno-2'-deoxyadenosine (1,N⁶-εdAdo); **c** 5-methyl-2'-deoxycytidine (5-mC), 5-hydroxymethyl-2'-deoxycytidine (5-hmC), and 2'-deoxycytidine (dCyd). The analyses were performed with multiple reaction monitoring (MRM) by using the fragmentations specified in the images for each analyte. The internal standards are represented by the red traces

control group exposed to ambient air, and both groups were treated inside the chambers of a Harvard Ambient Fine-Particle Concentrator located near the crossroad. The total intake dose (ID) of 70.2 μg (1.08 μg per day of exposure) was estimated according to Alexander et al. [85], with the inhalability of the particles determined according to Menache et al. [86] [ID = C × f × VT × I (d50%, 5g) × t, where C is the PM_{2.5} concentration (600 μg/m³), f is the breathing frequency (250 inhalations/min), VT is the tidal volume (0.15 mL), I is the inhalability (d50%, 5g = 80%), and t is the duration of exposure

(3900 min = 65 days, 5 days per week, 1 h per day)]. If mice were exposed to 20 μg/m³ PM_{2.5} for 24 h, then the estimated intake dose of particles would be 0.864 μg/day (56.16 μg after 65 days). Thus, we can assume that the adopted exposure protocol mimics a 24 h exposure to concentrations of ambient fine particulate matter found in São Paulo City.

The air pollutants data obtained from the Cerqueira César monitoring station of the São Paulo State Environmental Agency (CETESB, São Paulo, Brazil) for the exposure period of the present study (September 1 to



November 30, 2010) showed that the ambient air PM₁₀ and PM_{2.5} average (\pm SD) concentrations were $31.61 \pm 22.16 \mu\text{g}/\text{m}^3$ and $20.36 \pm 11.82 \mu\text{g}/\text{m}^3$, respectively [87]. The 2008–2010 triennium average (\pm SD) PM₁₀ and PM_{2.5} concentrations in the Cerqueira César monitoring station were $30.86 \pm 5.86 \mu\text{g}/\text{m}^3$ and $17.73 \pm$

$1.69 \mu\text{g}/\text{m}^3$, respectively [87]. Previous characterization of PM_{2.5} collected in the vicinity of the site of exposure showed that approximately 67% of the PM_{2.5} mass was traffic related [76, 88]. Elemental analysis of PM_{2.5} collected inside the concentrated PM_{2.5} exposure chamber also reinforces that vehicular traffic was the major

Table 5 Method accuracy and coefficient of variation (CV) for quantification of 8-oxodGuo, 1,N²-edGuo and 1,N⁶-edAdo in DNA

Basal level Average ± SD (fmol)	Added fmol	Detected Average ± SD (fmol)	Detected(-)Basal Average (fmol)	Accuracy %	CV %
8-oxodGuo					
373.00 ± 2.71	0	372.79 ± 50,60	–	–	13.57
373.98 ± 4.86	367	755.41 ± 107,92	381	103.93	14.29
374.84 ± 5.19	734	1069.57 ± 108,51	695	94.65	10.14
357.94 ± 15.05	1469	1671.67 ± 44,27	1314	89.43	2.65
371.07 ± 2.43	2204	2272.01 ± 40,20	1901	86.25	1.77
1,N ² -edGuo					
0.54 ± 0.01	0	0.54 ± 0.09	–	–	16.88
0.54 ± 0.01	1	1.47 ± 0.16	0.93	93.39	11.17
0.55 ± 0.01	5	5.30 ± 0.72	4.76	95.11	13.50
0.53 ± 0.01	10	10.60 ± 0.39	10.06	100.63	3.67
0.54 ± 0.01	20	20.20 ± 0.93	19.66	98.29	4.60
1,N ⁶ -edAdo					
2.08 ± 0.10	0	2.29 ± 0.39	–	–	17.05
2.05 ± 0.04	1	3.06 ± 0.47	1.01	100.89	15.31
1.99 ± 0.06	5	7.87 ± 1.66	5.88	117.60	21.10
2.03 ± 0.07	10	12.43 ± 1.25	10.41	104.06	10.06
1.97 ± 0.03	20	22.42 ± 3.89	20.46	102.29	17.34

source of PM_{2.5} in the present study (Table 1), as indicated by the presence of Ca, Mg, Zn, and Pb, which are mainly derived from the combustion of gasoline and oil additives; Zn, Pb, Cd and V, which are derived from diesel tailpipe emissions; and Sb, Fe, Ba, Cu and Al, which are derived from break wear [89]. Polycyclic aromatic hydrocarbons were also detected in PM_{2.5} collected at the site of exposure (Table 2).

The metal content and organic components of PM_{2.5} are important factors for the induction of DNA damage [90, 91]. By direct or indirect mechanisms, both groups of components may induce ROS formation, which, if not equilibrated by cell antioxidant systems, subsequently leads to an increased rate of generation of oxidatively damaged biomolecules [90–96]. A major DNA damage used as a marker of oxidative stress is 8-oxodGuo, a mutagenic lesion formed by the mono electronic oxidation of guanine, or by hydroxyl radical or singlet oxygen attack of guanine in DNA [97].

As reviewed by Møller and coworkers [98], there are few reports on the assessment of oxidatively damaged DNA in the lungs of animals exposed to air pollution particles, with little evidence for associations [98]. Typically, positive associations have been found after rodent exposure to high DEPs concentrations or doses (3 to 80 mg/m³, exposure by inhalation for few hours or 1 to 12 months; 0.1 to 4 mg by intratracheal instillation, analysis after a few hours or days) [98, 99]. The long-term inhalation of light-duty diesel engine exhaust (DEPs

3.5 mg/m³, 17 h/day, 3 days/week, for 1 to 12 months) led to the induction of 8-oxodGuo in rat lungs at a rate that was higher during the first 3 months of exposure, despite the continuous linear increase in the lung burden of deposited particles along the 12 months [100]. However, the short-term inhalation of a NIST standard reference material 1650 DEP (5 or 20 mg/m³, 1.5 h/day, 4 days) did not change the levels of 8-oxodGuo in mouse lungs, which were increased only after a single 1.5 h exposure to 80 mg/m³ DEP [101]. Contrary to wild-type mice, repair-deficient *Ogg1*^{-/-} mice exposed by inhalation to the NIST standard reference material 2975 DEP (20 mg/m³, 1.5 h/day, 4 days) presented increased lung levels of 8-oxodGuo [98]. Indeed, when the mice were exposed to DEP obtained from a diesel engine (0.05, 0.1 and 0.2 mg) for 10 weeks by weekly intratracheal administration, lung 8-oxodGuo levels were increased (for DEP 0.1 and 0.2 mg) and correlated with lung tumor incidence (for DEP 0.05 and 0.1 mg) [29].

Here, we showed that mice exposed for 3 months to ambient PM_{2.5} at a concentration that leads to the estimated total intake dose of 70.2 µg had increased levels of 8-oxodGuo in the lung tissue (Table 4). This is the first time that such an increase has been experimentally demonstrated for an estimated lung burden of particles close to what would be expected after 24 h/day exposure along 3 months to the average PM_{2.5} concentration found in the local ambient air.

Reported basal levels of 8-oxodGuo in rodent lung tissue, based on HPLC analyses, range from 180 to 450/10⁸ dGuo [44, 101–104], 1340 – 2120/10⁸ dGuo [100], or approximately 3000/10⁸ dGuo [29, 105], with the lowest values obtained from DNA extraction methods by using sodium iodide. The mean 8-oxodGuo level found here in the lung of mice exposed to ambient air was 2124/10⁸ dGuo. The level increased to 2466/10⁸ dGuo in the animals exposed to ambient air enriched in PM_{2.5} (Table 4).

An inter-laboratory assessment of 8-oxodGuo in DNA extracted from standard samples of pig liver and distributed by the European Standards Committee on Oxidative DNA Damage (ESCODD) to different research groups revealed a great discrepancy in the levels detected between laboratories, ranging from 223 to 44,100/10⁸ dGuo, with a median level of 1047/10⁸ dGuo [106]. In the present study, the mean 8-oxodGuo levels found in ambient air exposed mice lung, kidney, and liver DNA were, respectively, 2.0, 1.8, and 2.7 times higher than the median basal level obtained by ESCODD. Considering the limitations of 8-oxodGuo analyses, the DNA samples were extracted and stored by using solutions containing deferoxamine for protection against artifactual oxidation, and the hydrolysis procedure was performed by using 80 µg of DNA from each sample to minimize the contribution of spurious oxidation to the final result, as suggested by Helbock and coworkers [107].

It has been shown that human exposure to each 10 µg PM_{2.5}/m³ could result in an 11% increase in the levels of 8-oxodGuo in lymphocyte DNA [92]. Increased levels of urinary or serum 8-oxodGuo in humans were also associated with PM_{2.5} mass, its organic and/or elemental constituents, or the duration of exposure [91, 108–110]. Additionally, children exposed to high levels of a complex mixture of air pollutants in Mexico City presented higher levels of 8-oxodGuo in nasal biopsies from the posterior inferior turbinate compared to those of controls [111].

Consistent with an expected increased rate of generation of superoxide radical (O₂^{•-}) in the lung tissue of mice exposed to the concentrated ambient PM_{2.5}, lung SOD activity was increased in these animals (Table 3). In a previous study, mice intranasally instilled with a suspension of Residual Oil Fly Ashes (0.2 mg/kg), a surrogate for ambient air PM in many studies, and euthanized 1 or 3 h after exposure, presented increased lung oxygen consumption, NADPH oxidase activity, mitochondrial state 3 respiration, nitric oxide production, phospholipid oxidation, and carbonyl content [112]. Lung SOD activity was also increased in the reported model as a response to the amplified generation of O₂^{•-} by different sources (NADPH oxidase, mitochondria), favoring O₂^{•-} dismutation to H₂O₂ [103]. Catalase, glutathione peroxidases (GPx) and peroxiredoxins (Prxs) reduce H₂O₂ to water, decreasing the chance of its reduction to the

highly reactive hydroxyl radical ([•]OH) by transition metals (Fe²⁺, Cu⁺), and preventing the occurrence of oxidative damage [113–115]. While catalase directly decomposes H₂O₂ to H₂O and O₂, the thiol peroxidases GPx and Prxs reduce H₂O₂ to H₂O via simultaneous oxidation of glutathione (GSH) and thioredoxin (Trx). Oxidized glutathione and thioredoxin (GSSG e TrxS₂) are reduced by the NADPH-consuming enzymes glutathione reductase (GR) and thioredoxin reductase [113, 115, 116]. Here, the animals exposed to concentrated ambient PM_{2.5} had decreased GR activity in the lung (Table 3), which may unbalance an important defense system against the excess H₂O₂ from O₂^{•-} dismutation, enabling the increased generation of oxidative damage, as observed for 8-oxodGuo. The erythrocytes of the animals exposed to concentrated ambient PM_{2.5} also presented increased SOD activity. This finding is consistent with the knowledge that particles deposited in the lungs not only provoke lung inflammation but also induce a systemic inflammatory response, with the activation of NADPH oxidase in the systemic circulation leading to augmented O₂^{•-} generation [117].

Exposure to concentrated PM_{2.5} also led to increased levels of DNA lesions in internal organs, such as the liver (augmented 1,N²-εdGuo) and kidneys (augmented 8-oxodGuo and 1,N⁶-εdAdo). As the animals were exposed whole body to the pollutants, the different routes of absorption (pulmonary, gastrointestinal, and dermal) may contribute to these effects. The lesions 1,N²-εdGuo and 1,N⁶-εdAdo are formed by reactive carbonyls resulting from lipid peroxidation [32] and were demonstrated to play a role in the pathophysiology of inflammation [36, 37]. Rats exposed orally to carbon black (0.64 mg/kg b.w.) presented increased levels of 8-oxodGuo, 1,N⁶-εdAdo, and 1,N²-εdGuo in the liver, with the highest effect observed for 1,N²-εdGuo [44]. However, only 1,N²-εdGuo levels were increased in the livers of the animals exposed orally to PM (0.64 mg/kg b.w.) collected from a wood stove-rich area [44]. Thus, different profiles of DNA lesions may be induced in response to the exposure of different tissues to different pollutants. A more comprehensive approach quantifying a large panel of lesions will likely reveal yet unknown effects. The differences in these profiles may be influenced by the time the sample was collected, and the inflammatory condition, pollutant biotransformation, and repair capacity of the target tissues [44]. Decreased SOD and increased GST activities were observed in the livers of mice exposed to concentrated PM_{2.5} in the present study, showing a shift of the detoxification system towards the conjugation of reactive electrophiles with glutathione, consistent with the observed increased levels of 1,N²-εdGuo. The present study is the first to show the induction of etheno adducts in the liver and kidneys of mice exposed to ambient PM_{2.5}.

The levels of $1,N^6$ - ϵ dAdo detected in the present study fall within the range obtained in studies employing ultra-sensitive immunoaffinity/ 32 P-postlabeling and are lower than those described by other groups employing HPLC-ESI-MS/MS (Table 6). Similarly, the $1,N^2$ - ϵ dGuo levels quantified in the present study are consistent with the lowest levels reported by Garcia [118] and Angeli [119] by using HPLC-ESI-MS/MS (Table 6).

In addition to the genotoxic effect of PM, another important consequence of the exposure is the induction of epigenetic changes, which may affect gene expression without altering the nucleotide sequence in DNA [120–122]. Approximately 70% of the CpG dinucleotides in DNA are methylated, whereas non methylated CpGs are primarily found in “CpG islands”. The dense methylation of promoter regions is associated with gene silencing [123–128].

Here, we observed that $PM_{2.5}$ exposure led to decreased global levels of 5-hmC in lung (34% decrease) and liver (16% decrease) DNA, without affecting the global levels of 5-mC (Table 4). This observation points to global 5-hmC as a more sensitive biomarker. Oxidative stress is a possible contributing factor to the decreased 5-hmC levels. The effect of oxidative stress decreasing the global levels of 5-hmC and modulating the genomic hydroxymethylation profile was demonstrated by Delatte and coworkers [129] in SY5Y human neuroblastoma cell line exposed to buthionine sulfoximine, and in colon epithelia of mice depleted for *GPx1* and *GPx2*, both situations leading to increased levels of peroxides. We observed a significant inverse correlation between 5-hmC and 8-oxodGuo in mice lung DNA (Fig. 3a). Additionally, several lines of evidence indicate changes in DNA methylation and

hydroxymethylation as key events favoring cancer development due to benzo[*a*]pyrene exposure [27, 130–133]. Benzo[*a*]pyrene was one of the PAHs present in the $PM_{2.5}$ collected at the site of exposure (Table 2). Diminished 5-hmC levels were also observed in human bronchial epithelial cells (HBECs) exposed to DEPs (5 mg/cm², 24 h) in vitro [69] and in buccal cells of humans exposed to increased concentrations of $PM_{2.5}$ or PM_{10} [65].

As 5-hmC is a first step in the DNA demethylation pathway catalyzed by TET [57], the decrease in its levels may represent decreased TET activity or increased 5-hmC removal from DNA. Previous studies have suggested that the loss of TET activity may lead to the hypermethylation of gene promoter regions, with the consequent deregulation of gene transcription and cell differentiation [128]. Here we observed that the 5-mC/5-hmC ratio increased in lung DNA of the $PM_{2.5}$ exposed mice (Fig. 3b). Differentiated cells in stratified epithelia of several human and mouse organs presented higher levels of 5-hmC than the stem/progenitor cells and cancer cells [134]. The loss of 5-hmC was suggested to be an early event in carcinogenesis [134].

Ding and coworkers [66] evaluated for the first time the effects of traffic-related air pollution on DNA methylation in rat lung. The authors found, using multiple linear regression, that exposure to $PM_{2.5}$, PM_{10} and NO_2 was associated with changes in DNA methylation (decreased methylation of *LINE1* and *iNOS* promoter, and increased promoter methylation of *APC* and *p16^{CDKN2A}*) [66]. However, the different exposure localities chosen for the control and exposed groups prevented them from ruling out “the effects from the stress induced by other factors” [66]. In the present study, we focused on the effects of $PM_{2.5}$

Table 6 Levels of $1,N^2$ - ϵ dGuo and $1,N^6$ - ϵ dAdo in DNA from biological samples in different studies

Species/Model	Tissue	Method	Level	References
$1,N^2$ - ϵ dGuo				
Human cell line IMR-90	Lung	LC-ESI-MS/MS	0.2–1.8/10 ⁷ dGuo	[118]
Human cell line SW480	Colon adenocarcinoma	LC-ESI-MS/MS	0.5–2/10 ⁷ dGuo	[119]
Human cell line A549	Lung	LC-ESI-MS/MS	1.5–5/10 ⁶ dGuo	[45]
Rat	Liver	LC-ESI-MS/MS	1–3.25/10 ⁶ dGuo	[44]
Rat	Lung	LC-ESI-MS/MS	1.5–2.5/10 ⁶ dGuo	[44]
$1,N^6$ - ϵ dAdo				
Human	Lung	32 P-postlabeling	2.4–146/10 ⁹ dAdo	[139]
Human cell line A549	Lung	LC-ESI-MS/MS	0.1–0.45/10 ⁶ dGuo	[45]
Human	White Blood Cells	32 P-postlabeling	0.08–24.07/10 ⁸ dAdo	[140]
Human	White Blood Cells	32 P-postlabeling	138–1017/10 ⁹ dAdo	[141]
Human	White Blood Cells	32 P-postlabeling	0.03–90.15/10 ⁷ dAdo	[142]
Rat	Liver	LC-ESI-MS/MS	0.75–2/10 ⁶ dGuo	[44]
Rat	Liver	LC-ESI-MS/MS	2.1–6.9/10 ⁸ tdn	[143]
Rat	Lung	LC-ESI-MS/MS	0.5–1.25/10 ⁶ dGuo	[44]

tdn total deoxynucleosides

concentrated from the ambient air, compared to the ambient air exposed group submitted, in the same place and at the same time, to equal conditions of manipulation and stress, except the concentration of PM_{2.5}. This is the first assessment of the levels of DNA lesions, 5-mC, 5-hmC, and oxidative stress in different tissues of mice in such an experimental condition, allowing direct evidence of the effects of PM_{2.5} as it occurs in the environment. However, some short-comings of using the ambient air exposed group as the control must be addressed. The endpoints quantified in this group are not the basal levels that could be present in an unexposed control group. Studies using a clean air site or HEPA filtration in situ to prevent any exposure of the control group to PM would improve the sensitivity for detection of differences between groups.

The assessment of the total levels of 5-mC and 5-hmC in DNA is usually a first step in approaches to understand the effects of different agents on the epigenome, as pointed out by Bakulski and Fallin [52]. Although the total 5-mC and 5-hmC levels in DNA do not give information about the genes affected, this type of analysis provides useful data for the direction of greater resolution studies. For example, (1) detecting changes using the whole DNA denotes a broad effect, which probably encompasses different genes; (2) if the investigated agents affect the epigenetic machinery (e.g., TETs, DNMTs, pathways required to recycle the co-substrates of these enzymes, etc.), changes of the total 5-mC and/or 5-hmC levels are expected [69, 121, 133, 135–137]; (3) a broad effect is more likely to replicate in different animal species, compared to specific changes in genes that depend on different factors, including host development and genetics [121, 129, 133]. On the other hand, if no change is observed in the total levels of 5-mC or 5-hmC, biologically important changes across the genome cannot be excluded. The gain and loss of 5-mC or 5-hmC in different genomic locations may be counter-balanced, resulting in no net change of the global levels, which must be interpreted with caution [135].

Jiang and coworkers [133] assessed the DNA methylation profiles of immortalized human bronchial epithelial cells and murine skin exposed to benzo[*a*]pyrene in vitro and in vivo, respectively. It was evident the broad effect of benzo[*a*]pyrene modulating the DNA methylation in both systems (2414 differentially methylated regions in the exposed murine skin; 105,958 and 20,577 differentially methylated sites in two exposed human cell lines). A total of 153 genes with altered promoter methylation (hypermethylation or hypomethylation) were found in the exposed murine skin, 45 of them (29%) occurred in the exposed human cells. In contrast, 10,447 genes with altered promoter methylation were detected in the exposed human cells [133]. Delatte and coworkers [129] provided the opportunity to compare differentially hydroxymethylated genes induced by oxidative stress in SY5Y human neuroblastoma cell line and in colon

epithelia of mice. The global levels of 5-hmC were decreased in both systems under oxidative stress, but the genes and pathways altered, although involved in the oxidative stress response, differed between the systems [129]. Studies comparing the specific agent-induced changes in DNA methylation and hydroxymethylation profiles in tissues of animal models, human cells and human tissues would allow a better understanding of the applicability of such detailed information obtained in an animal model.

LINE1 and Alu repeat elements have been used as surrogate assays for quantifying total 5-mC in the majority of the studies on associations between ambient PM exposure and global DNA methylation alterations [47, 49, 58, 59, 61, 62, 66, 67]. Other methods used for quantification of 5-mC and/or 5-hmC in studies of PM exposure were ELISA [68, 69], indirect assays [63], HPLC-UV [51], HPLC-ESI-MS/MS [64, 65]. LINE1 and Alu assays do not distinguish between 5-mC and 5-hmC, and limit the assessment of methylation levels to specific cytosines in bisulfite converted DNA, quantified by pyrosequencing after polymerase chain reaction [135]. Conversely, HPLC based methods allow the direct quantification of the total levels of DNA bases, including 5-mC and 5-hmC separately, and are then considered the “gold standard” for this purpose [135]. A community-wide benchmarking study compared several methods for DNA methylation analysis [136]. Among the six global DNA methylation assays included in the study (HPLC-MS, ELISA, bisulfite pyrosequencing of four repetitive elements), HPLC-MS data most accurately reflected the expected differences between samples [136].

Using HPLC based methods, De Prins and coworkers [51] found that increased exposure to NO₂, PM₁₀, PM_{2.5} and O₃ was associated with decreased total 5-mC levels in human blood DNA; Janssen and coworkers [64] observed that total 5-mC levels in human placenta were inversely associated with PM_{2.5} exposure; and Nys and coworkers [65] were the first to show an association between increased human exposure to PM_{2.5} and PM₁₀ and decreased 5-mC and 5-hmC total levels in human buccal cell DNA. So far, to the best of our knowledge, this study is the first to quantify 5-mC and 5-hmC by HPLC-ESI-MS/MS in different tissues (lung, liver and kidney) of mice selectively exposed to ambient PM_{2.5}. Our observations encourage further investigation of the effects of PM_{2.5} exposure on the profiles of the genomic methylation and hydroxymethylation of target tissues to obtain a better understanding of the deregulated molecular pathways that may culminate in disease (e.g., cancer, metabolic disorders, cardiovascular disease) development [138].

Conclusions

Mice exposed daily, for 3 months, to PM_{2.5} at a concentration that mimics 24-h exposure to the mean concentration

found in ambient air, presented altered antioxidant system activity in lung, liver, and erythrocytes, increased levels of DNA lesions related to oxidative stress in lung, liver, and kidney, and decreased global levels of 5-hmC in lung and liver DNA. This is the first direct demonstration of the occurrence of these effects due to exposure to PM_{2.5} as it occurs in the environment. Genetic and epigenetic alterations induced by pollutants may affect the genes committed to cell cycle control, apoptosis, and cell differentiation, increasing the chance of cancer development, which merits further investigation.

Additional file

Additional file 1: Table S1. Reagents used in the study. **Table S2.** Parameters used in the ESI-MS/MS equipment for detection of the lesions and epigenetic marks in DNA. **Figure S1.** System of two columns used for 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) analyses. A) Configuration used in the first 16 min and from 32 to 46 min of the chromatography; B) Configuration used in the interval 16 – 32 min, allowing further separation and peak narrowing in column B prior to elution to the ESI source of the mass spectrometer. **Figure S2.** Calibration curves obtained by HPLC-ESI-MS/MS for quantification of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo), 1,N²-etheno-2'-deoxyguanosine (1,N²-edGuo), 1,N⁶-etheno-2'-deoxyadenosine (1,N⁶-edAdo), 2'-deoxycytidine (dCyd), 5-methyl-2'-deoxycytidine (5-mC), and 5-hydroxymethyl-2'-deoxycytidine (5-mC, lower range). (DOCX 738 kb)

Abbreviations

1,N²-edGuo: 1,N²-etheno-2'-deoxyguanosine; 1,N⁶-edAdo: 1,N⁶-etheno-2'-deoxyadenosine; 5-hmC: 5-hydroxymethylcytosine; 5-mC: 5-methylcytosine; 8-oxodGuo: 8-oxo-7,8-dihydro-2'-deoxyguanosine; 8-oxoGua: 8-oxo-7,8-dihydro-guanine; APC: Adenomatous polyposis coli; CAT: Catalase; CDNB: 1-chloro-2,4-dinitrobenzene; CETESB: São Paulo State Environmental Agency; dAdo: 2'-deoxyadenosine; dCyd: 2'-deoxycytidine; DEP: Diesel exhaust particles; dGuo: 2'-deoxyguanosine; DNMT: DNA methyltransferase; ESCODD: European Standards Committee on Oxidative DNA Damage; ESI: Electrospray ionization; GPx: Glutathione peroxidase; GR: Glutathione reductase; GSH: Reduced glutathione; GSSG: Oxidized glutathione; GST: Glutathione S-transferase; HBECs: Human bronchial epithelial cells; HPLC: High performance liquid chromatography; IARC: International Agency for Research on Cancer; iNOS: Inducible nitric oxide synthase; LINE1: Long interspersed nucleotide element; LOD: Limit of detection; LOQ: Limit of quantitation; *m/z*: mass-to-charge ratio; MRM: Multiple reaction monitoring; MS: Mass spectrometry; NADPH: Nicotinamide adenine dinucleotide phosphate; NIST: National Institute of Standards and Technology; Ogg1: 8-oxoguanine DNA glycosylase; PAH: Polycyclic aromatic hydrocarbon; PDA: Photodiode array detector; PM: Particulate matter; Prxs: Peroxiredoxins; ROS: Reactive oxygen species; S/N: Signal-to-noise ratio; SD: Standard deviation; SEM: Standard error of the mean; SOD: Superoxide dismutase; TET: Ten-eleven-translocation; Trx: Thioredoxin

Acknowledgements

Prof. Mary Rosa Rodrigues de Marchi, Departamento de Química Analítica, Instituto de Química, Universidade Estadual Paulista (UNESP) for performing the PAHs analysis. MSc Rosana Astolfo and Prof. Maria de Fátima Andrade Lapat - Laboratório de Análises de Processos Atmosféricos, Instituto de Astronomia, Geofísica e Ciências Atmosféricas, Universidade de São Paulo (USP) for the PM_{2.5} elemental analysis.

Funding

FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, Proc. 2012/22190-3 and 2012/08616-8), CNPq (Proc. 454214/2014-6), CAPES, PRPUSP (Pró-Reitoria de Pesquisa da Universidade de São Paulo), INCT INAIRA (MCT/CNPq/FNDCT/CAPES/FAPEMIG/FAPERJ/FAPESP; Proc. 573813/2008-6), INCT Redoxoma (FAPESP/CNPq/CAPES; Proc. 573530/2008-4), NAP

Redoxoma (PRPUSP; Proc. 2011.1.9352.1.8) and CEPID Redoxoma (FAPESP; Proc. 2013/07937-8). T. F. Oliveira and A. A. F. Oliveira received scholarships from FAPESP (Proc. 2012/21636-8, 2011/09891-0, 2012/08617-4) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). M. H. G. Medeiros, P. Di Mascio, T. Marcourakis, P. H. N. Saldiva, and A. P. M. Loureiro received fellowships from CNPq.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

AAFO and TFO conducted experiments, performed data analyses and interpretation, drafted the manuscript, and prepared the figures and tables. MD, ML and MMV performed experiments and data analyses. MHGM, PDM and TM performed data interpretation. PHNS designed the study and performed data interpretation. APML designed the study, performed data interpretation, and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval

The experimental protocol for this study was approved by the Ethics Committee of the Faculty of Medicine, University of São Paulo (protocol n^o 1310/09).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

¹Departamento de Análises Clínicas e Toxicológicas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Av. Prof. Lineu Prestes 580, Bloco 13 B, São Paulo CEP 05508-000, Brazil. ²Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Av. Prof. Lineu Prestes 748, São Paulo CEP 05508-000, Brazil. ³Laboratório de Poluição Atmosférica Experimental – LIM05, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, Av. Dr. Arnaldo 455, São Paulo CEP 01246903, Brazil. ⁴Instituto de Estudos Avançados, Universidade de São Paulo, R. do Anfitéatro, 513, São Paulo CEP 05508060, Brazil. ⁵Present address: Departamento de Farmacociências, Universidade Federal de Ciências da Saúde de Porto Alegre, Rua Sarmento Leite 245, Porto Alegre, Rio Grande do Sul CEP 90050-170, Brazil.

Received: 9 April 2018 Accepted: 3 October 2018

Published online: 19 October 2018

References

- Kok TM, Hogervorst JG, Briedé JJ, Van Herwijnen MH, Maas LM, Moonen EJ, et al. Genotoxicity and physicochemical characteristics of traffic-related ambient particulate matter. *Environ Mol Mutagen.* 2005;46:71–80.
- Pope CA III, Dockery DW. 2006 critical review: health effects of fine particulate air pollution: lines that connect. *J Air Waste Manage Assoc.* 2006; 56:709–42.
- Meng Z, Zhang Q. Damage effects of dust storm PM_{2.5} on DNA in alveolar macrophages and lung cells of rats. *Food Chem Toxicol.* 2007;45:1368–74.
- Møller P, Folkmann JK, Forchhammer L, Bräuner EV, Danielsen PH, Risom L, et al. Air pollution, oxidative damage to DNA, and carcinogenesis. *Cancer Lett.* 2008;266:84–97.
- Demetriou CA, Raaschou-Nielsen O, Loft S, Møller P, Vermeulen R, Palli D, et al. Biomarkers of ambient air pollution and lung cancer: a systematic review. *Occup Environ Med.* 2012;69:619–27.
- Benbrahim-Tallaa L, Baan RA, Grosse Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, et al. Carcinogenicity of diesel-engine and gasoline-engine exhausts and some nitroarènes. *Lancet Oncol.* 2012;13:663–4.
- Yanagi Y, de Assunção JV, Barrozo LV. The impact of atmospheric particulate matter on cancer incidence and mortality in the city of São Paulo, Brazil. *Cad Saúde Pública.* 2012;28:1737–48.

8. DeMarini DM. Genotoxicity biomarkers associated with exposure to traffic and near-road atmospheres: a review. *Mutagenesis*. 2013;28:485–505.
9. Fajersztajn L, Veras M, Barrozo LV, Saldiva P. Air pollution: a potentially modifiable risk factor for lung cancer. *Nat Rev Cancer*. 2013;13:674–8.
10. IARC. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Outdoor Air Pollution, vol. 109. Lyon: IARC; 2016. p. 448.
11. Raaschou-Nielsen O, Andersen ZJ, Beelen R, Samoli E, Stafoggia M, Weinmayr G, et al. Air pollution and lung cancer incidence in 17 European cohorts: prospective analyses from the European study of cohorts for air pollution effects (ESCAPE). *Lancet Oncol*. 2013;14:813–22.
12. Carvalho VSB, Freitas ED, Martins LD, Martins JA, Mazzoli CR, de Fátima Andrade M. Air quality status and trends over the metropolitan area of Sao Paulo, Brazil as a result of emission control policies. *Environ Sci Pol*. 2015;47:68–79.
13. CETESB - Companhia Ambiental do Estado de São Paulo. Qualidade do Ar no Estado de São Paulo 2013. 2013. <http://cetesb.sp.gov.br/ar/qualar/>. Accessed 29 Oct 2017.
14. Anderson LG. Ethanol fuel use in Brazil: air quality impacts. *Energy Environ Sci*. 2009;2:1015.
15. Nogueira T, de Souza KF, Fornaro A, Andrade M de F, de Carvalho LRF. On-road emissions of carbonyls from vehicles powered by biofuel blends in traffic tunnels in the metropolitan area of Sao Paulo, Brazil. *Atmos Environ*. 2015;108:88–97.
16. World Health Organization. WHO Air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulfur dioxide. Global Update 2005. Summary of Risk Assessment. 2005. http://apps.who.int/iris/bitstream/10665/69477/1/WHO_SDE_PHE_OEH_06.02_eng.pdf. Accessed 12 Mar 2018.
17. Brook RD, Rajagopalan S, Pope CA, Brook JR, Bhatnagar A, Diez-Roux AV, et al. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American heart association. *Circulation*. 2010;121:2331–78.
18. Martins LD, Martins JA, Freitas ED, Mazzoli CR, Gonçalves FLT, Ynoue RY, et al. Potential health impact of ultrafine particles under clean and polluted urban atmospheric conditions: a model-based study. *Air Qual Atmos Health*. 2010;3:29–39.
19. Samet JM. Some current challenges in research on air pollution and health. *Salud Públ Méx*. 2014;56:379–85.
20. Dybdahl M, Risom L, Bornholdt J, Autrup H, Loft S, Wallin H. Inflammatory and genotoxic effects of diesel particles in vitro and in vivo. *Mutat Res Genet Toxicol Environ Mutagen*. 2004;562:119–31.
21. Riva DR, Magalhães CB, Lopes AA, Lanças T, Mauad T, Malm O, et al. Low dose of fine particulate matter (PM_{2.5}) can induce acute oxidative stress, inflammation and pulmonary impairment in healthy mice. *Inhal Toxicol*. 2011;23:257–67.
22. Möller P, Folkmann JK, Danielsen PH, Jantzen K, Loft S. Oxidative stress generated damage to DNA by gastrointestinal exposure to insoluble particles. *Curr Mol Med*. 2012;12:732–45.
23. De Martinis BS, Kado NY, Carvalho LRF, Okamoto RA, Gundel LA. Genotoxicity of fractionated organic material in airborne particles from São Paulo, Brazil. *Mutat Res*. 1999;446:83–94.
24. Karlsson HL, Nygren J, Möller L. Genotoxicity of airborne particulate matter: the role of cell-particle interaction and of substances with adduct-forming and oxidizing capacity. *Mutat Res Genet Toxicol Environ Mutagen*. 2004;565:1–10.
25. Bell ML, Dominici F, Ebisu K, Zeger SL, Samet JM. Spatial and temporal variation in PM_{2.5} chemical composition in the United States for health effects studies. *Environ Health Perspect*. 2007;115:989–95.
26. DeMarini DM, Landi S, Tian D, Hanley NM, Li X, Hu F, et al. Lung tumor *KRAS* and *TP53* mutations in nonsmokers reflect exposure to PAH-rich coal combustion emissions. *Cancer Res*. 2001;61:6679–81.
27. Chappell G, Pogribny IP, Guyton KZ, Rusyn I. Epigenetic alterations induced by genotoxic occupational and environmental human chemical carcinogens: a systematic literature review. *Mutat Res*. 2016;768:27–45.
28. Ke S, Liu Q, Yao Y, Zhang X, Sui G. An in vitro cytotoxicities comparison of 16 priority polycyclic aromatic hydrocarbons in human pulmonary alveolar epithelial cells HPAEpiC. *Toxicol Lett*. 2018;290:10–8.
29. Ichinose T, Yajima Y, Nagashima M, Takenoshita S, Nagamachi Y, Sagai M. Lung carcinogenesis and formation of 8-hydroxy-deoxyguanosine in mice by diesel exhaust particles. *Carcinogenesis*. 1997;18:185–92.
30. Loft S, Svoboda P, Kawai K, Kasai H, Sørensen M, Tjønneland A, et al. Association between 8-oxo-7,8-dihydroguanine excretion and risk of lung cancer in a prospective study. *Free Radic Biol Med*. 2012;52:167–72 Elsevier Inc.
31. Obtulowicz T, Swoboda M, Speina E, Gackowski D, Rozalski R, Siomek A, et al. Oxidative stress and 8-oxoguanine repair are enhanced in colon adenoma and carcinoma patients. *Mutagenesis*. 2010;25:463–71.
32. Marnett LJ, Plataras JP. Endogenous DNA damage and mutation. *Trends Genet*. 2001;17:214–21.
33. Bartsch H, Nair J. New DNA-based biomarkers for oxidative stress and cancer chemoprevention studies. *Eur J Cancer*. 2000;36:1229–34.
34. Doerge DR, Churchwell MI, Fang JL, Beland FA. Quantification of etheno-DNA adducts using liquid chromatography, on-line sample processing, and electrospray tandem mass spectrometry. *Chem Res Toxicol*. 2000;13:1254–9.
35. Loureiro APM, Marques SA, Garcia CCM, Di Mascio P, Medeiros MHG. Development of an on-line liquid chromatography-electrospray tandem mass spectrometry assay to quantitatively determine 1,N²-etheno-2'-deoxyguanosine in DNA. *Chem Res Toxicol*. 2002;15:1302–8.
36. Nair U, Bartsch H, Nair J. Lipid peroxidation-induced DNA damage in cancer-prone inflammatory diseases: a review of published adduct types and levels in humans. *Free Radic Biol Med*. 2007;43:1109–20.
37. Pang B, Zhou X, Yu H, Dong M, Taghizadeh K, Wishnok JS, et al. Lipid peroxidation dominates the chemistry of DNA adduct formation in a mouse model of inflammation. *Carcinogenesis*. 2007;28:1807–13.
38. Medeiros MHG. Exocyclic DNA adducts as biomarkers of lipid oxidation and predictors of disease. Challenges in developing sensitive and specific methods for clinical studies. *Chem Res Toxicol*. 2009;22:419–25.
39. Garcia CCM, Freitas FP, Di Mascio P, Medeiros MHG. Ultrasensitive simultaneous quantification of 1,N²-Etheno-2'-deoxyguanosine and 1,N²-Propano-2'-deoxyguanosine in DNA by an online liquid chromatography electrospray tandem mass spectrometry assay. *Chem Res Toxicol*. 2010;23:1851.
40. Chen HJ, Lin WP. Quantitative analysis of multiple exocyclic DNA adducts in human salivary dna by stable isotope dilution nanoflow liquid chromatography - nanospray ionization tandem mass spectrometry. *Anal Chem*. 2011;83:8543–51.
41. Garcia CCM, Freitas FP, Sanchez AB, Di Mascio P, Medeiros MHG. Elevated α -methyl- γ -hydroxy-1,N²-propano-2'-deoxyguanosine levels in urinary samples from individuals exposed to urban air pollution. *Chem Res Toxicol*. 2013;26:1602–4.
42. Cui S, Li H, Wang S, Jiang X, Zhang S, Zhang R, et al. Ultrasensitive UPLC-MS-MS method for the quantitation of etheno-DNA adducts in human urine. *Int J Environ Res Public Health*. 2014;11:10902–14.
43. Monien BH, Schumacher F, Herrmann K, Glatt H, Turesky RJ, Chesne C. Simultaneous detection of multiple DNA adducts in human lung samples by isotope-dilution UPLC-MS/MS. *Anal Chem*. 2015;87:641–8.
44. Danielsen PH, Loft S, Jacobsen NR, Jensen KA, Autrup H, Ravanat JL, et al. Oxidative stress, inflammation, and DNA damage in rats after intratracheal instillation or oral exposure to ambient air and wood smoke particulate matter. *Toxicol Sci*. 2010;118:574–85.
45. Danielsen PH, Moller P, Jensen KA, Sharma AK, Wallin H, Bossi R, et al. Oxidative stress, DNA damage, and inflammation induced by ambient air and wood smoke particulate matter in human A549 and THP-1 cell lines. *Chem Res Toxicol*. 2011;24:168–84.
46. Knaapen AM, Güngör N, Schins RPF, Borm PJA, Van Schooten FJ. Neutrophils and respiratory tract DNA damage and mutagenesis: a review. *Mutagenesis*. 2006;21:225–36.
47. Bellavia A, Urch B, Speck M, Brook RD, Scott JA, Albetti B, et al. DNA hypomethylation, ambient particulate matter, and increased blood pressure: findings from controlled human exposure experiments. *J Am Heart Assoc*. 2013;2:1–11.
48. Chen W-L, Lin C-Y, Yan Y-H, Cheng KT, Cheng T-J. Alterations in rat pulmonary phosphatidylcholines after chronic exposure to ambient fine particulate matter. *Mol Biosyst*. 2014;10:3163–9.
49. Baccarelli A, Wright RO, Bollati V, Tarantini L, Litonjua AA, Suh HH, et al. Rapid DNA methylation changes after exposure to traffic particles. *Am J Respir Crit Care Med*. 2009;179:572–8.
50. Feil R, Fraga MF. Epigenetics and the environment: emerging patterns and implications. *Nat Rev Genet*. 2012;13:97–109.
51. De Prins S, Koppen G, Jacobs G, Dons E, Van de Mierop E, Nelen V, et al. Influence of ambient air pollution on global DNA methylation in healthy adults: a seasonal follow-up. *Environ Int*. 2013;59:418–24.
52. Bakulski KM, Fallin MD. Epigenetic epidemiology: promises for public health research. *Environ Mol Mutagen*. 2014;55:171–83.
53. Robertson KD, Wolffe AP. DNA methylation in health and disease. *Nat Rev Genet*. 2000;1:11–9.
54. Bender J. Dna methylation and epigenetics. *Annu Rev Plant Biol*. 2004;55:41–68.

55. Magaña AA, Wrobel K, Caudillo YA, Zaina S, Lund G, Wrobel K. High-performance liquid chromatography determination of 5-methyl-2'-deoxycytidine, 2'-deoxycytidine, and other deoxynucleosides and nucleosides in DNA digests. *Anal Biochem.* 2008;374:378–85.
56. Smith ZD, Meissner A. DNA methylation: roles in mammalian development. *Nat Rev Genet.* 2013;14:204–20.
57. Branco MR, Ficz G, Reik W. Uncovering the role of 5-hydroxymethylcytosine in the epigenome. *Nat Rev Genet.* 2012;13:7–13.
58. Tarantini L, Bonzini M, Apostoli P, Pegoraro V, Bollati V, Marinelli B, et al. Effects of particulate matter on genomic DNA methylation content and iNOS promoter methylation. *Environ Health Perspect.* 2009;117:217–22.
59. Madrigano J, Baccarelli A, Mittleman MA, Wright RO, Sparrow D, Vokonas PS, et al. Prolonged exposure to particulate pollution, genes associated with glutathione pathways, and DNA methylation in a cohort of older men. *Environ Health Perspect.* 2011;119:977–82.
60. Guo L, Byun H-M, Zhong J, Motta V, Barupal J, Zheng Y, et al. Effects of short-term exposure to inhalable particulate matter on DNA methylation of tandem repeats. *Environ Mol Mutagen.* 2014;55:322–35.
61. Chen R, Meng X, Zhao A, Wang C, Yang C, Li H, et al. DNA hypomethylation and its mediation in the effects of fine particulate air pollution on cardiovascular biomarkers: a randomized crossover trial. *Environ Int.* 2016;94:614–9.
62. Alvarado-Cruz I, Sánchez-Guerra M, Hernández-Cadena L, Vizcaya-Ruiz A, Mugica V, Pelallo-Martínez NA, et al. Increased methylation of repetitive elements and DNA repair genes is associated with higher DNA oxidation in children in an urbanized, industrial environment. *Mutat Res.* 2017;813:27–36.
63. Yauk C, Polyzos A, Rowan-Carroll A, Somers CM, Godschalk RW, Van Schooten FJ, et al. Germ-line mutations, DNA damage, and global hypermethylation in mice exposed to particulate air pollution in an urban/industrial location. *Proc Natl Acad Sci.* 2008;105:605–10.
64. Janssen BG, Godderis L, Pieters N, Poels K, Kiciński M, Cuypers A, et al. Placental DNA hypomethylation in association with particulate air pollution in early life. *Part Fibre Toxicol.* 2013;10:22.
65. Nys S, Duca R, Nawrot T, Hoet P, Meerbeek BV, Landuyt KLV, et al. Temporal variability of global DNA methylation and hydroxymethylation in buccal cells of healthy adults: association with air pollution. *Environ Int.* 2018;111:301–8.
66. Ding R, Jin Y, Liu X, Zhu Z, Zhang Y, Wang T, et al. Characteristics of DNA methylation changes induced by traffic-related air pollution. *Mutat Res.* 2016;796:46–53.
67. Cai J, Zhao Y, Liu P, Xia B, Zhu Q, Wang X, et al. Exposure to particulate air pollution during early pregnancy is associated with placental DNA methylation. *Sci Total Environ.* 2017;607–608:1103–8.
68. Sanchez-Guerra M, Zheng Y, Osorio-Yanez C, Zhong J, Chervona Y, Wang S, et al. Effects of particulate matter exposure on blood 5-hydroxymethylation: results from the Beijing truck driver air pollution study. *Epigenetics.* 2015;10:633–42.
69. Sominen HK, Zhang X, Biagini Myers JM, Kovacic MB, Ulm A, Jurcak N, et al. Ten-eleven translocation 1 (TET1) methylation is associated with childhood asthma and traffic-related air pollution. *J Allergy Clin Immunol.* 2016;137:797–805.e5.
70. Mangal D, Vudathala D, Park J-H, Lee SH, Penning TM, Blair IA. Analysis of 7,8-Dihydro-8-oxo-2'-deoxyguanosine in cellular DNA during oxidative stress. *Chem Res Toxicol.* 2009;22:788–97.
71. Loureiro APM, Di Mascio P, Gomes OF, Medeiros MHG. trans,trans-2,4-Decadienal-induced 1,N2-Etheno-2'-deoxyguanosine adduct formation. *Chem Res Toxicol.* 2000;13:601–9.
72. Hilleström PR, Weimann A, Poulsen HE. Quantification of urinary etheno-DNA adducts by column-switching LC/APCI-MS/MS. *J Am Soc Mass Spectrom.* 2006;17:605–10.
73. Hofer T, Seo AY, Prudencio M, Leeuwenburgh C. A method to determine RNA and DNA oxidation simultaneously by HPLC-ECD: greater RNA than DNA oxidation in rat liver after doxorubicin administration. *Biol Chem.* 2006;387:103–11.
74. Sandhu J, Kaur B, Armstrong C, Talbot CJ, Steward WP, Farmer PB, et al. Determination of 5-methyl-2'-deoxycytidine in genomic DNA using high performance liquid chromatography-ultraviolet detection. *J Chromatogr B Anal Technol Biomed Life Sci.* 2009;877:1957–61.
75. Kim S, Sioutas C, Chang MC, Gong H. Factors affecting the stability of the performance of ambient fine-particle concentrators. *Inhal Toxicol.* 2000;12(Suppl 4):281–98.
76. Akinaga LMY, Lichtenfels AJ, Carvalho-Oliveira R, Caldini EG, Dolhnikoff M, Silva LFF, et al. Effects of chronic exposure to air pollution from Sao Paulo city on coronary of Swiss mice, from birth to adulthood. *Toxicol Pathol.* 2009;37:306–14.
77. Veras MM, Damaceno-Rodrigues NR, Guimarães Silva RM, Scoriza JN, Saldiva PHN, Caldini EG, et al. Chronic exposure to fine particulate matter emitted by traffic affects reproductive and fetal outcomes in mice. *Environ Res.* 2009;109:536–43.
78. Magalhães D, Bruns RE, de C Vasconcelos P. Hidrocarbonetos policíclicos aromáticos como traçadores da queima de cana-de-açúcar: uma abordagem estatística. *Quim Nova.* 2007;30:577–81.
79. Flöhé L, Günzler WA. Assays of glutathione peroxidase. *Methods Enzymol.* 1984;105:114–21.
80. Carlberg I, Mannervik B. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. *J Biol Chem.* 1975;250:5475–80.
81. Habig WH, Pabst MJ, Fleischner G, Gatmaitan Z, Arias IM, Jakoby WB. The identity of glutathione S-transferase B with ligandin, a major binding protein of liver. *Proc Natl Acad Sci U S A.* 1974;71:3879–82.
82. McCord JM, Fridovich I. The utility of superoxide dismutase in studying free radical reactions. I. Radicals generated by the interaction of sulfite, dimethyl sulfoxide, and oxygen. *J Biol Chem.* 1969;244:6056–63.
83. Flöhé L, Otting F. Superoxide dismutase assays. *Methods Enzymol.* 1984;105:93–104.
84. Aebi H. Catalase in vitro. *Methods Enzymol.* 1984;105:121–6.
85. Alexander DJ, Collins CJ, Coombs DW, Gilkison IS, Hardy CJ, Healey G, et al. Association of inhalation toxicologists (AIT) working party recommendation for standard delivered dose calculation and expression in non-clinical aerosol inhalation toxicology studies with pharmaceuticals. *Inhal Toxicol.* 2008;20(13):1179–89.
86. Ménache MG, Miller FJ, Raabe OG. Particle inhalability curves for humans and small laboratory animals. *Ann Occup Hyg.* 1995;39(3):317–28.
87. CETESB - Companhia Ambiental do Estado de São Paulo. *Qualar – Sistema de Informações da Qualidade do Ar* (2010). <http://cetesb.sp.gov.br/ar/qualar/>. Accessed 29 Oct 2017.
88. Mauad T, Rivero DHRF, De Oliveira RC, Lichtenfels AJDFC, Guimarães ET, De Andre PA, et al. Chronic exposure to ambient levels of urban particles affects mouse lung development. *Am J Respir Crit Care Med.* 2008;178:721–8.
89. Schauer JJ, Lough GC, Shafer MM, Christensen WF, Arndt MF, DeMinter JT, et al. Characterization of metals emitted from motor vehicles. *Res Rep Health Eff Inst.* 2006;133:77–88.
90. Gutiérrez-Castillo ME, Roubicek DA, Cebrián-García ME, De Vizcaya-Ruiz A, Sordo-Cedeño M, Ostrosky-Wegman P. Effect of chemical composition on the induction of DNA damage by urban airborne particulate matter. *Environ Mol Mutagen.* 2006;47:199–211.
91. Huang HB, Lai CH, Chen GW, Lin YY, Jaakkola JJK, Liou SH, et al. Traffic-related air pollution and DNA damage: a longitudinal study in Taiwanese traffic conductors. *PLoS One.* 2012;7:1–8.
92. Sørensen M, Autrup H, Hertel O, Wallin H, Knudsen LE, Loft S. Personal exposure to PM_{2.5} and biomarkers of DNA damage. *Cancer Epidemiol Biomark Prev.* 2003;12:191–6.
93. Rossner P, Topinka J, Hovorka J, Milcova A, Schmuczerova J, Krouzek J, et al. An acellular assay to assess the genotoxicity of complex mixtures of organic pollutants bound on size segregated aerosol. Part II: oxidative damage to DNA. *Toxicol Lett.* 2010;198:312–6.
94. Hou L, Zhang X, Zheng Y, Wang S, Dou C, Guo L, et al. Altered methylation in tandem repeat element and elemental component levels in inhalable air particles. *Environ Mol Mutagen.* 2014;55:256–65.
95. Hellack B, Quass U, Beuck H, Wick G, Kuttler W, Schins RPF, et al. Elemental composition and radical formation potency of PM₁₀ at an urban background station in Germany in relation to origin of air masses. *Atmos Environ.* 2015;105:1–6.
96. Valavanidis A, Fiotakis K, Bakeas E, Vlahogianni T. Electron paramagnetic resonance study of the generation of reactive oxygen species catalysed by transition metals and quinoid redox cycling by inhalable ambient particulate matter. *Redox Rep.* 2005;10:37–51.
97. Cadet J, Loft S, Olinski R, Evans MD, Bialkowski K, Richard Wagner J, et al. Biologically relevant oxidants and terminology, classification and nomenclature of oxidatively generated damage to nucleobases and 2-deoxyribose in nucleic acids. *Free Radic Res.* 2012;46:367–81.
98. Møller P, Danielsen PH, Karotki DG, Jantzen K, Roursgaard M, Klingberg H, et al. Oxidative stress and inflammation generated DNA damage by exposure to air pollution particles. *Mutat Res Rev Mutat Res.* 2014;762:133–66.
99. Risom L, Møller P, Loft S. Oxidative stress-induced DNA damage by particulate air pollution. *Mutat Res Mol Mech Mutagen.* 2005;592:119–37.
100. Iwai K, Adachi S, Takahashi M, Moller L, Udagawa T, Mizuno S, et al. Early oxidative DNA damages and late development of lung cancer in diesel exhaust-exposed rats. *Environ Res.* 2000;84:255–64.

101. Risom L, Dybdahl M, Bornholdt J, Vogel U, Wallin H, Møller P, et al. Oxidative DNA damage and defence gene expression in the mouse lung after short-term exposure to diesel exhaust particles by inhalation. *Carcinogenesis*. 2003;24:1847–52.
102. Risom L, Dybdahl M, Møller P, Wallin H, Haug T, Vogel U, et al. Repeated inhalations of diesel exhaust particles and oxidatively damaged DNA in young oxoguanine DNA glycosylase (OGG1) deficient mice. *Free Radic Res*. 2007;41:172–81.
103. Tsurudome Y, Hirano T, Yamato H, Tanaka I, Sagai M, Hirano H, et al. Changes in levels of 8-hydroxyguanine in DNA, its repair and OGG1 mRNA in rat lungs after intratracheal administration of diesel exhaust particles. *Carcinogenesis*. 1999;20:1573–6.
104. Marie-Desvergne C, Maitre A, Bouchard M, Ravanat JL, Viau C. Evaluation of DNA adducts, DNA and RNA oxidative lesions, and 3-hydroxybenzo(a)pyrene as biomarkers of DNA damage in lung following intravenous injection of the parent compound in rats. *Chem Res Toxicol*. 2010;23:1207–14.
105. Schmerold I, Niedermu H. Levels of 8-hydroxy-2'-deoxyguanosine in cellular DNA from 12 tissues of young and old Sprague Dawley rats. *Exp Gerontol*. 2001;36:1375–86.
106. ESCODD (European Standards Committee on Oxidative DNA Damage). Comparative analysis of baseline 8-oxo-7,8-dihydroguanine in mammalian cell DNA, by different methods in different laboratories: an approach to consensus. *Carcinogenesis*. 2002;23:2129–33.
107. Helbock HJ, Beckman KB, Shigenaga MK, Walter PB, Woodall AA, Yeo HC, et al. DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. *Proc Natl Acad Sci*. 1998;95:288–93.
108. Kim JY, Mukherjee S, Ngo L, Christiani DC. Urinary 8-hydroxy-2'-deoxyguanosine as a biomarker of oxidative DNA damage in workers exposed to fine particulates. *Environ Health Perspect*. 2004;112:666–71.
109. Wei Y, Han I, Shao M, Hu M, Zhang JJ, Tang X. PM 2.5 constituents and oxidative DNA damage in humans. *Environ Sci Technol*. 2009;43:4757–62.
110. Tan C, Lu S, Wang Y, Zhu Y, Shi T, Lin M, et al. Long-term exposure to high air pollution induces cumulative DNA damages in traffic policemen. *Sci Total Environ*. 2017;593–594:330–6.
111. Calderón-Garcidueñas L, Wen-Wang L, Zhang YJ, Rodriguez-Alcaraz A, Osnaya N, Villarreal-Calderón A, et al. 8-hydroxy-2'-deoxyguanosine, a major mutagenic oxidative DNA lesion, and DNA strand breaks in nasal respiratory epithelium of children exposed to urban pollution. *Environ Health Perspect*. 1999;107:469–74.
112. Magnani ND, Marchini T, Tasat DR, Alvarez S, Evelson PA. Lung oxidative metabolism after exposure to ambient particles. *Biochem Biophys Res Commun*. 2011;412:667–72.
113. Halliwell B, Gutteridge JMC. *Free radicals in biology and medicine*. 4th ed. New York: Oxford University Press; 2007.
114. Bartosz G. Reactive oxygen species: destroyers or messengers? *Biochem Pharmacol*. 2009;77:1303–15.
115. Forman HJ, Ursini F, Maiorino M. An overview of mechanisms of redox signaling. *J Mol Cell Cardiol*. 2014;73:2–9.
116. Lee S, Kim SM, Lee RT. Thioredoxin and thioredoxin target proteins: from molecular mechanisms to functional significance. *Antioxid Redox Signal*. 2013;18:1165–207.
117. Kampfrath T, Maisseyeu A, Ying Z, Shah Z, Deiluiis JA, Xu X, et al. Chronic fine particulate matter exposure induces systemic vascular dysfunction via NADPH oxidase and TLR4 pathways. *Circ Res*. 2011;108:716–26.
118. Garcia CCM, Angeli JPF, Freitas FP, Gomes OF, de Oliveira TF, Loureiro APM, et al. [13C2]-acetaldehyde promotes unequivocal formation of 1,N2-propano-2'-deoxyguanosine in human cells. *J Am Chem Soc*. 2011;133:9140–3.
119. Angeli JPF, Garcia CCM, Sena F, Freitas FP, Miyamoto S, Medeiros MHG, et al. Lipid hydroperoxide-induced and hemoglobin-enhanced oxidative damage to colon cancer cells. *Free Radic Biol Med*. 2011;51:503–15.
120. Herceg Z, Vaissière T. Epigenetic mechanisms and cancer: an interface between the environment and the genome. *Epigenetics*. 2011;6:804–19.
121. Ji H, Khurana Hershey GK. Genetic and epigenetic influence on the response to environmental particulate matter. *J Allergy Clin Immunol*. 2012;129:33–41.
122. Silveyra P, Floros J. Air pollution and epigenetics: effects on SP-A and innate host defence in the lung. *Swiss Med Wkly*. 2012;142:w13579.
123. Irizarry RA, Ladd-Acosta C, Wen B, Wu Z, Montano C, Onyango P, et al. The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. *Nat Genet*. 2009;41:178–86.
124. Doi A, Park I-H, Wen B, Murakami P, Aryee MJ, Irizarry R, et al. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nat Genet*. 2009;41:1350–3.
125. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. *Carcinogenesis*. 2010;31:27–36.
126. Brenet F, Moh M, Funk P, Feierstein E, Viale AJ, Socci ND, et al. DNA methylation of the first exon is tightly linked to transcriptional silencing. *PLoS One*. 2011;6:e14524 Papavasiliou N, editor.
127. Dahl C, Grønbaek K, Guldborg P. Advances in DNA methylation: 5-hydroxymethylcytosine revisited. *Clin Chim Acta*. 2011;412:831–6.
128. Williams K, Christensen J, Helin K. DNA methylation: TET proteins-guardians of CpG islands? *EMBO Rep*. 2011;13:28–35.
129. Delatte B, Jeschke J, Defrance M, Bachman M, Creppe C, Calonne E, et al. Genome-wide hydroxymethylcytosine pattern changes in response to oxidative stress. *Sci Rep*. 2015;5:12714.
130. Damiani LA, Yingling CM, Leng S, Romo PE, Nakamura J, Belinsky SA. Carcinogen-induced gene promoter hypermethylation is mediated by DNMT1 and causal for transformation of immortalized bronchial epithelial cells. *Cancer Res*. 2008;68:9005–14.
131. Teneng I, Montoya-Durango DE, Quartermous JL, Lacy ME, Ramos KS. Reactivation of L1 retrotransposon by benzo(a)pyrene involves complex genetic and epigenetic regulation. *Epigenetics*. 2011;6:355–67.
132. Xia B, Yang L-Q, Huang H-Y, Pang L, Yang X-F, Yi Y-J, et al. Repression of biotin-related proteins by benzo[a]pyrene-induced epigenetic modifications in human bronchial epithelial cells. *Int J Toxicol*. 2016;35:336–43.
133. Jiang C-L, He S-W, Zhang Y-D, Duan H-X, Huang T, Huang Y-C, et al. Air pollution and DNA methylation alterations in lung cancer: a systematic and comparative study. *Oncotarget*. 2017;8:1369–91.
134. Haffner MC, Chau A, Meeker AK, Esopi DM, Gerber J, Pellakuru LG, et al. Global 5-hydroxymethylcytosine content is significantly reduced in tissue stem/progenitor cell compartments and in human cancers. *Oncotarget*. 2011;2:627–37.
135. Lisanti S, Omar WA, Tomaszewski B, De Prins S, Jacobs G, Koppen G, et al. Comparison of methods for quantification of global DNA methylation in human cells and tissues. *Plos One*. 2013;8:e79044.
136. Bock C, Halbritter F, Carmona FJ, Tierling S, Datlinger P, Assenov Y, et al. Quantitative comparison of DNA methylation assays for biomarker development and clinical applications. *Nat Biotechnol*. 2016;34:726–40.
137. Tammen SA, Dolnikowski GG, Ausman LM, Liu Z, Sauer J, Friso S, et al. Aging and alcohol interact to Alter hepatic DNA Hydroxymethylation. *Alcohol Clin Exp Res*. 2014;38:2178–85.
138. Jin Z, Liu Y. DNA methylation in human diseases. *Gene Dis*. 2018;5:1–8.
139. Godshalk R, Nair J, van Schooten FJ, Risch A, Drings P, Kayser K, et al. Comparison of multiple DNA adduct types in tumor adjacent human lung tissue: effect of cigarette smoking. *Carcinogenesis*. 2002;23:2081–6.
140. Dechakhamphu S, Pinalor S, Sithithaworn P, Nair J, Bartsch H, Yongvanit P. Lipid peroxidation and etheno DNA adducts in white blood cells of liver fluke-infected patients: protection by plasma alpha-tocopherol and praziquantel. *Cancer Epidemiol Biomark Prev*. 2010;19:310–8.
141. Arab K, Pedersen M, Nair J, Meerang M, Knudsen LE, Bartsch H. Typical signature of DNA damage in white blood cells: a pilot study on etheno adducts in Danish mother-newborn child pairs. *Carcinogenesis*. 2009;30:282–5.
142. Nair J, Vaca CE, Velic I, Mutanen M, Valsta LM, Bartsch H. High dietary omega-6 polyunsaturated fatty acids drastically increase the formation of etheno-DNA base adducts in white blood cells of female subjects. *Cancer Epidemiol Biomark Prev*. 1997;6:597–601.
143. Churchwell MI, Beland FA, Doerge DR. Quantification of multiple DNA adducts formed through oxidative stress using liquid chromatography and electrospray tandem mass spectrometry. *Chem Res Toxicol*. 2002;15:1295–301.