Published in final edited form as:
Mutat Res. 2008 December 8; 657(2): 116–121. doi:10.1016/j.mrgentox.2008.08.015.

Particulate matter inhibits DNA repair and enhances mutagenesis

Manju Mehta, **Lung-Chi Chen**, **Terry Gordon**, **William Rom**, and **Moon-shong Tang***
Departments of Environmental Medicine, Pathology and Medicine, New York University School of Medicine, Tuxedo, New York 10987

Abstract

Exposure to ambient air pollution has been associated with adverse health effects including lung cancer. A recent epidemiology study has established that each 10 μg/m^3 elevation in long term exposure to average $PM_{2.5}$ ambient concentration was associated with approximately 8% of lung cancer mortality. The underlying mechanisms of how PM contributes to lung carcinogenesis, however, remain to be elucidated. We have recently found that transition metals such as nickel and chromium and oxidative stress induced lipid peroxidation metabolites such as aldehydes can greatly inhibit nucleotide excision repair (NER) and enhance carcinogen-induced mutations. Because PM is rich in metal and aldehyde content and can induce oxidative stress, we tested the effect of PM on DNA repair capacity in cultured human lung cells using *in vitro* DNA repair synthesis and host cell reactivation assays. We found that PM greatly inhibits NER for ultraviolet (UV) light and benzo(a)pyrene diol epoxide (BPDE) induced DNA damage in human lung cells. We further demonstrated that PM exposure can significantly increase both spontaneous and UV-induced mutagenesis. These results together suggest that the carcinogenicity of PM may act through its combined effect on suppression of DNA repair and enhancement of DNA replication errors.

Keywords

Particulate matter; Nucleotide excision repair (NER); Mutagenesis; Host cell reactivation (HCR)

Introduction

Epidemiological studies have long recognized increased mortality and morbidity in susceptible populations during high air pollution episodes [1,2]. This finding suggests a strong relationship between particulate matter (PM) and impairment of cardiopulmonary function [3]. Airborne PM is a complex mixture of particles that varies not only in size and morphology, but also in their chemical, physical, and biological characteristics. PM is

*Corresponding author. Tel.: +1 845 731 3585; fax: +1 845 351 2385; tang@env.med.nyu.edu.

usually described in terms of aerodynamic size as ≤ 10μm (PM_{10}) or ≤ 2.5μm ($PM_{2.5}$). $PM_{2.5}$ is dominated by products of combustion and secondary aerosols, while $PM_{2.5–10}$ consists mainly of crustal, biological and mechanically-derived particle fraction components [4]. PM of different aerodynamic sizes have a varying ability to penetrate the respiratory system; i.e., fine particles reach the deeper regions of the lungs, whereas coarse PM may be deposited in upper regions of the airways [5].

A recent epidemiology study has established that each 10 mg/m^3 increment in long-term exposure to ambient $PM_{2.5}$ is associated with approximately 8% of lung cancer mortality [6]. The underlying mechanisms of how PM contributes to lung carcinogenesis, however, remain to be understood. Since many carcinogens and co-carcinogens found in tobacco smoke (TS), such as polycyclic aromatic hydrocarbons (PAHs), transition metals, fatty acids, and aldehydes, are present in $PM_{2.5}$, which also contains ultra-fine fraction of PM (smaller than ~0.1 μm) that originates largely from combustion and secondary atmospheric chemistry processes, it is reasonable to hypothesize that these components contribute to PM-related lung carcinogenesis, as they do in TS-induced lung carcinogenesis [7]. Metabolically activated PAHs are able to damage DNA, induce mutations and initiate carcinogenesis [8–10]. Transition metals, although poor mutagens and carcinogens by themselves, can enhance the mutagenicity and carcinogenicity of other carcinogens such as PAHs [11]. It has been shown that PM exposure generates not only reactive oxygen species (ROS), but also oxidative DNA damage such as 8-oxo-deoxyguanine (8-oxo-dG) [12]. A recent study has shown that 8-oxo-dG are the most abundant DNA adducts induced by PM [13]. These results strongly suggest that PM causes a significant increase in oxidative stress in cells. This elevated oxidative stress has been attributed to the transition metals in PM that may facilitate Fenton reactions and generate ROS as well as the metabolism of aryl hydrocarbons by detoxification enzymes that also generate ROS [13]. It has also been demonstrated that the components in PM can induce bulky DNA adducts and oxidative bases that may induce mutations. Indeed, ample evidence has demonstrated that ambient pollutants are able to induce DNA adducts, mutations and tumors in animal models [14,15]. Although the half-life of ROS is extremely short, ROS can react with different cellular components, particularly fatty acids, generating various products including aldehydes in reaction called lipid peroxidation (LPO). Aldehydes have longer half-lives than ROS and are very reactive with thiol-containing proteins in cells [16]. Moreover, it is likely that the function of aldehyde-adducted proteins will be impaired.

We have recently found that both heavy metals and aldehydes can greatly inhibit nucleotide excision repair (NER) and consequently enhance the mutagenicity of various carcinogens [17,18]. NER is the major DNA repair pathway in human cells and repairs most bulky DNA adducts induced endogenously as well as by exogenous sources. It has been well established that individuals with genetic defects in repair genes, such as xeroderma pigmentosum (XP), suffer a 80 to 90% reduction in NER and have a 2000-fold increased incidence of skin cancer and 10 to 20-fold increased incidence of cancer of internal organs [19,20]. These findings led us to hypothesize that the reduction of DNA repair capacity is an important mechanism for carcinogens and cocarcinogens to cause mutations and subsequently cancer. Since PM contains transition metals (https://srmors.nist.gov/view_detail.cfm?srm=1649A) and aldehydes [21,22] that we have shown can inhibit NER, it is possible that PM may

inhibit DNA repair and this effect may contribute to lung carcinogenesis. In this study, we tested the effect of PM on DNA repair capacity in cultured human cells using *in vitro* DNA repair synthesis and host cell reactivation (HCR) assays [18]. We used size fractionated PM (SRM 1649a) collected from Washington D.C. area by National Institute of Technology (NIST). We found that this PM greatly inhibits NER for ultraviolet (UV) light and benzo(a)pyrene diol epoxide (BPDE) induced DNA damage in human lung cells. We further demonstrated that PM exposure could significantly increase both spontaneous and UV-induced mutagenesis. These results together suggest that the carcinogenicity of PM may act through its effect on suppression of DNA repair and enhancement of DNA replication errors.

Materials and Methods

Preparation of fine/ultra-fine (F/U) particles

Fine/ultra-fine (F/U) particles were prepared from urban dust 1649a purchased from National Institute of Standards and Technology (NIST), Gaithersburg, MD, using a Micro-orifice uniform deposit impactor (MOUDI) model no. 100 (rotating) (MSP corporation, Minneapolis, MN) operated at 30 L/min; the method was the same as previously described [23]. The reason for choosing this dust for this study was that its composition has been thoroughly determined. Particles were collected in discrete size ranges by passing the aerosol through a number of stages in series, with each subsequent stage collecting particles smaller than the one before it. The F/U fraction was collected on the after-filter, which is a PTFE (polytetrafluoroethylene) membrane with a PMP (polymehtylpentene) ring (Pall Corporation, East Hills, NY) with a cut-off size of 0.18 μm. The amount of PM collected on the PTFE filters were determined by weighing the filters before and after particle collection in a temperature and humidity controlled weighing room.

Preparation of PM suspension

The membrane part of filter was removed by cutting out the plastic rim using sterile forceps and scissors. PM suspension was then prepared by sonicating the serum free medium containing the filter using a Branson Sonifier 450 (20 pulses at #3 output control and 30% duty cycle). The total suspended particles (TSP), the original non-size fractionated 1649a, suspension was prepared by mixing the weighed amount of TSP with serum free medium.

Cells culture and cell viability assay

Human lung adenocarcinoma cells (A549) (American Type Culture Collection, Manassas, VA) were grown in DMEM medium supplemented with 10% fetal bovine serum. Logarithmically growing A549 cells were subjected to treatment with various concentrations of the TSP or the F/U fraction of PM (0.18 μm and lower) in DMEM (Dulbecco's modified eagle's medium) for 24 h at 37°C. Cell toxicity was examined in two ways. First, treated cells were rinsed with PBS (phosphate buffered saline, 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4, pH 7.4), immediately trypsinized and treated with trypan blue dye, and examined under light microscope as previously described [18]. Second, to determine colony-forming ability, the trypsinized cells were seeded (400 cells/dish) in fresh complete culture medium. After 10 days of incubation, colonies were fixed with methanol,

stained with crystal violet and counted [17]. Colony formation ability was calculated based on the plating efficiency of treated cells versus the plating efficiency of untreated control cells.

Host cell reactivation (HCR) assay

The pGL3-luciferase plasmid (Promega) was irradiated with UV light (germicidal lamp, >95% emission at 254 nm) at 1500 J/m^2 or modified with BPDE (15 mM) for 30 min as previously described [24]. A549 cells were plated in triplicate in 60 mm dishes at a density of 3×10^5 cells/dish and exposed to DMEM media alone or various concentrations of PM in DMEM medium at 37 °C for 24 h. After treatment, cells were rinsed with PBS, and then transfected with pGL3-luciferase reporter plasmid irradiated with UV or modified with BPDE. The transfection was performed for 16 h using Fugene 6. The untreated pSV-β -galactosidase control vector, a β-galactosidase-expressing plasmid, was co-transfected into cells to normalize transfection efficiency. Cells were further incubated with PM at pre-treatment concentration in complete growth medium for 24 h and then lysed with reporter lysis buffer (Promega). Transient expression of luciferase and β-galactosidase was determined as described [11]. Values of luciferase expression were normalized to the β-galactosidase and averaged over the triplicates. Since the reporter gene is not expressed unless DNA damage induced by UV or BPDE is repaired by cells, this assay can be used to detect the repair capacity of cells. The relative luciferase activity from UV- or BPDE-treated pGL3-lucifearse reporter plasmids was expressed as a percentage of luciferase activity from untreated pGL3-luciferase reporter plasmids and used to represent the repair capacity of cells. The relative repair capacity of cells was calculated as the percentage of the relative luciferase activity of the plasmids transfected into PM treated cells as compared to untreated cells.

Preparation of cell extract for DNA repair synthesis

Logarithmically growing cells were treated with various concentrations of F/U PM in DMEM medium for 24 h at 37 °C. Cells were rinsed with PBS and harvested immediately after treatment. Whole cell extracts were prepared according to the methods of Wood RD *et. al.* [25] with the exception that the concentration of DTT in all DTT-containing buffers used for the preparation of cell extracts was 0.1 mM. The resultant cell extracts were quick-frozen in small aliquots and stored at −80°C. The protein content was determined by the Bio-Rad protein assay kit (Bio-Rad).

In vitro DNA repair synthesis assay

The pUC18 plasmid DNA was purified by CsCl density gradient centrifugation. The supercoiled plasmid DNA was further purified by 5 to 20% sucrose gradient centrifugation [25,26]. The supercoiled pUC18 plasmid DNA was then irradiated with 1500 J/m^2 UV. After modification, the supercoiled pUC18 plasmid DNA was purified again by 5 to 20% sucrose gradient centrifugation. The *in vitro* DNA repair synthesis assay was performed in 50 μl reaction mixtures containing 0.3 μg supercoiled untreated or UV treated pUC18 DNA, 45 mM HEPES-KOH (pH 7.8), 70 mM KCl, 50 μM DTT, 0.4 mM EDTA, 2 mM ATP, 20 μM each of dGTP, dCTP and TTP, 2μCi [α-^{32} P]dATP (3000 Ci/mmol), 40 mM

phosphocreatine, 2.5 μg creatine phosphokinase (Type 1, Sigma), 3.4 % glycerol, 18 μg bovine serum albumin and 80 μg of extract protein [25,26]. Reactions were carried out at 30 °C for 3 h and stopped by adding EDTA (final concentration 20 mM). The reaction mixtures were treated with 80 μg/ml RNAase A for 10 min at 37 °C and followed by 0.5 % sodium dodecyl sulfate and 0.2 mg/ml proteinase K treatment for 30 min at 37°C. Proteins in the reaction mixtures were removed by phenol/chloroform extractions and the plasmid DNA was ethanol precipitated, dissolved in TE buffer (Tris buffer 10 mM, PH, 7.5, and 1 mM EDTA) and then linearized by Hind III digestion. The linearized DNA was then separated by electrophoresis on a 1% agarose gel containing 0.5 μg/ml ethidium bromide. The gel was first photographed, dried and exposed to a Cyclone Phosphor screen (Packard). The amount of radioactive nucleotide incorporation in the DNA was quantified and then normalized with the DNA content measured by Bioimager. The amount of DNA repair synthesis was expressed as incorporation of [α-^{32}P]dATP per unit DNA.

SupF mutagenesis assay

A549 cells were grown to 70% confluence in 150 mm tissue-culture dishes, treated with different concentrations of F/U PM (0, 50, 100 and 200 μg/ml) at 37 °C for 24 h in the culture medium. Particles were then removed and the pSP189 plasmids with and without UV (1500 J/m^2) modifications were transfected into the cells as described previously[11]. After transfection, medium containing the transfection mixture was removed and cells were cultured in fresh medium in the presence of PM at the pre-treatment concentrations for another 60 h. The transfected plasmids were then rescued from the human cells by the alkaline lysis method [27]. DNA was then extracted with phenol and chloroform, precipitated with ethanol, resuspended in TE and then treated with the *Dpn*I restriction enzyme (New England Biolabs, Beverly, MA) to remove the unreplicated plasmids, which bear the bacterial adenine methylation pattern. The replicated plasmids were then electroporated into indicator MBM7070 bacteria, which carry a *lac*Z gene with an amber mutation [28]. The transformed bacteria were plated on LB plates containing ampicillin (50 μg/ml), isopropyl β-D-thiogalactoside (IPTG) (190 μg/ml), and 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) (0.8 mg/ml). After overnight incubation at 37°C, white and light blue mutant colonies were picked from the background of blue wild-type colonies and restreaked plasmids were then extracted and purified using the QIApre-spin plasmid kit (Qiagen, Valencia, CA). The sequences of the *supF* gene of mutant plasmids were determined with the primer 5'-GGC GAC ACG GAA ATG TTG AA-3'.

Results

PM is cytotoxic

Since PM contains numerous substances which are potentially genotoxic and cytotoxic in nature, we wished to determine the PM toxicity to human cells. Cultured human lung cells A549 were exposed to different concentrations of PM for 24 h and the viability of these cells was determined by both Trypan Blue dye exclusion assay and colony-forming ability assay. Results in Figure 1 show that PM is cytotoxic demonstrated by both assays, and that colony-forming assay is much more sensitive than the dye exclusion assay to detect PM cytotoxicity. The reduction of colony forming ability of PM exposed cells was proportional

to PM concentration. Exposure to 50 μg/ml PM caused a 10 % reduction of colony forming ability.

PM exposure causes an inhibitory effect on repair of UV- and BPDE-induced DNA damage in cultured human lung cells

Previously we have found that heavy metals, such as nickel and chromium, and aldehydes such as acrolein, malondialdehyde, and 4-hydroxy-2-nenonal can cause an inhibitory effect on human lung cells (7,11,17,18,31). Urban dust 1649a is rich in Ni and Cr content. Although the aldehydescontent in this dust was not analyzed, it has been found that dust collected from urban area of New York City, Los Angeles, and Elizabeth city New Jersey are rich in aldehydes [21,22]. These results raise the possibilities that urban dust can cause an inhibitory effect on DNA repair and this effect may contribute to lung carcinogenesis. To test this possibility we determined the effect of PM treatment on DNA repair capacity using HCR and DNA damage-dependent repair synthesis assays. In the HCR assay, plasmid containing a luciferase reporter gene was irradiated with UV or modified with BPDE and then transfected into cells pretreated with different concentrations of PM (0, 50, 100, 200, and 400 μg/ml, 24 h at 37 °C). Because the reporter gene will not be expressed unless UV-induced cyclobutane pyrimidine dimers or BPDE-DNA adducts in this gene are repaired by cells, the luciferase activity therefore represents the extent of repair which in turn reflects the cellular DNA repair capacity. The results in Figure 2 show that cells treated with total suspended particles (TSP) or F/U PM have reduced HCR capacity and this reduction of HCR is PM dose dependent. Furthermore, compared to TSP, F/U PM showed a greater inhibitory effect on HCR.

The slope of relative repair capacity for BPDE-DNA damage versus PM dose for TSP is −0.0776 and for F/U is −0.1663; the difference between these two slopes is statistically significant (*t*-ratio=10.98 with 26 degrees of freedom, $p<0.0001$). The slope of relative repair capacity for UV-DNA damage versus PM dose for TSP is −0.0868 and for F/U is −0.1410; the difference between these two slopes is also statistically significant (*t*-ratio=6.68 with 26 degrees of freedom $p<0.0001$). It should be noted that both UV- and BPDE-induced DNA damage is repaired by the same NER mechanism in human cells [32]; these results indicate that both TSP and F/U PM affect the same NER mechanism.

To further demonstrate the inhibitory effect on DNA repair by PM, using *in vitro* DNA damage-dependent repair synthesis assay we determined NER repair capacity in cell extracts prepared from A549 cells treated with F/U PM. Results in Figure 3 show that repair synthesis capacity was reduced in cells exposed to PM and this reduction in repair synthesis is also proportional to the PM concentration.

PM treatment enhances both spontaneous and UV-induced mutagenesis

PM treatment causes an inhibitory effect on NER; this effect may lead to enhancement of bulky DNA damage-induced mutagenesis since NER is the major pathway for repair of bulky DNA lesions in cells. To test this possibility we chose to use a shuttle vector containing *supF* to determine the effect of PM treatment on UV irradiation-induced mutagenesis in cultured lung cells. Shuttle vectors with and without UV irradiation were

transfected into cells pretreated with different concentrations of PM. After 60 h of incubation the replicated shuttle vectors were isolated and the number of mutations in *supF* was determined and the sequence changes in the mutated *supF* were also determined. The results in Figure 4 show that PM treatment significantly enhances UV-induced mutations. The majority of mutation sites in UV-irradiated *supF* are in sequences that have contiguous pyrimidines in one of the DNA strands (Fig. 5). Since the major UV-induced DNA damage are cyclobutane pyrimidine dimers and pyrimidine<6–4>pyrimidones, this result indicates that PM treatment enhances DNA damage-induced mutagenesis (target mutagenesis). Since PM treatment reduces NER capacity, we conclude that the enhancement of UV mutagenesis in PM treated cells is mainly due to inefficient repair of UV lesions in these cells. PM treatment also induces mutation frequency in undamaged *supF*; this effect could be originated from PM-induced inhibition on DNA repair of spontaneous DNA damage and/or reduction of DNA replication fidelity.

Discussion

There is an increasing body of evidence linking PM with elevated risk of lung cancer [1]. An epidemiological study demonstrated a strong link between fine particulate air pollution and elevated lung cancer mortality [29]. Because PM contains numerous direct and indirect DNA damaging agents such as PAHs, Cr and aldehydes, and ROS inducing metals such as Cr, Ni and arsenic, DNA damage induced by these agents in PM potentially contributes to lung carcinogenesis. These agents have also been found to inhibit cellular repair capacity and enhance mutagenesis [11,17]. It is well established that cells from individuals carrying genetic defects in DNA repair have a high spontaneous and DNA damaging agent-induced mutation frequency and these individuals suffer high cancer incidence [30]. This finding led us to hypothesize that reduction of DNA repair capacity, due to exogenous agents or endogenous metabolites, can enhance mutagenesis and therefore, carcinogenesis [7,17,31]. In this study, we have found that urban dust can indeed inhibit NER and enhance both spontaneous and UV-induced mutagenesis. We, therefore, propose that a PM-induced reduction of DNA repair contributes to PM-related lung cancer.

We found that human cells exposed to 100 μg/ml of the urban PM preparation (SRM 1649a) collected over a 12 month period in Washington D.C. area for 24 h has only a 10 % reduction in viability, but a 35 % reduction in repair capacity, and a 5-fold increase in mutation frequency. Safe limit standards set by the EPA for a 24 h exposure for fine particles and PM_{10} are 35 and 150 $\mu g/m^3$, respectively. This translates to a daily dose of 420 μg per day and 1800 μg per day fine PM and PM_{10}, respectively (based on a 12 m^3/day inhaled volume estimate). If this translation is correct then it is possible that some ill effects may be resulted from long term exposing to this safe limit.

Our results show that F/U PM has a more profound effect on DNA repair than TSP. The process of collecting F/U PM involves sampling the resuspended TSP with the MOUDI impactor with the F/U PM being collected at the last filter stage. Typically we start with about 250 mg of TSP and the amount collected on the MOUDI after-filter is about 2.5 mg. Assuming the recovery for F/U PM by this process is 100% then concentration of F/U PM in TSP is about 1 %. Our results show that 400 μg/ml of TSP causes about 30 % decrease in

DNA repair capacity; if we assume DNA repair inhibition solely results from F/U PM then only a 10 % drop in DNA repair capacity is expected. It is thus evident from Figure 2 that major effect of TSP on repair capacity comes from particles other than F/U particles. This effect, we suspect, is derived from dissolved metals and aldehydes of TSP.

How do metals and aldehydes in PM inhibit DNA repair? The initial step of NER events is the recognition of DNA lesions, which is accomplished by preferential binding of multiple proteins to the damaged DNA [32]. It has been reported that Ni and cadmium can reduce repair protein-DNA damage complex formation [33,34]. Aldehydes, Cr, and Ni are known to have a high affinity towards thiol groups and histidines and, therefore, their potential targets could be zinc finger structures in DNA binding motifs. Zinc finger proteins are a family of proteins in which zinc is complexed through four invariant cysteine and/or histidine residues forming a zinc finger domain, which is predominantly involved in DNA binding as well as with protein-protein interactions [35]. Human and *E. coli* NER protein XPA and UvrA both contain a single zinc finger although the function of this zinc finger is not clear [36,37]. It is possible that the replacement of zinc with other metals such as Ni (II) or adduction of cysteine/histidine by aldehydes at the zinc finger could dramatically affect the function and stability of these proteins. If PM inhibitory effect on NER is through these direct metal- and aldehydes-repair protein interactions then the levels of repair proteins in cells will determine the PM effect on cellular susceptibility repair capacity.

It should be noted a human lung adenocarcinoma cell line (A549) is used in this study. It is possible that PM may have different level of inhibitory effect on NER in normal human epithelial cells if the levels of repair proteins in these cells are different from lung tumor cells.

We have shown that PM reduces NER efficiency and, as expected, that PM enhances UV-induced mutagenesis. However, PM also enhances spontaneous mutation frequency. This enhancement could be due to inhibition of repair for spontaneous DNA damage or induced by PM treatment. It is worth noting that there were two significant different features of UV-induced mutation spectrum in cells treated with PM from cells without PM treatment: PM enhanced single base substitutions and greatly increased tandem mutations (Table 1). These results suggest that besides inhibiting DNA repair, PM may increase error in translesion synthesis. Previously, we have shown that Ni treatment enhances UV and BPDE-induced mutagenesis but does not change the mutation spectrum, and furthermore, Ni treatment does not enhance spontaneous mutation frequency [11]. These results together suggest that PM components that increase error in translesion synthesis do not include Ni.

In summary, we have found that PM inhibits NER and enhances both spontaneous and DNA damage-induced mutagenesis. We propose that these effects are derived from three mechanisms: 1) PM components such as heavy metals and aldehyde directly modify repair proteins and DNA, 2) ROS and secondary products of ROS produced by heavy metal-induced Fenton reaction modify repair protein and DNA, and 3) direct modifications of DNA replication proteins by heavy metals and aldehydes reduce the fidelity of DNA replication. We further propose that these effects contribute to lung carcinogenesis.

Acknowledgments

This work was supported by grants CA114541, ES03124 and ES014614 (M.-s. Tang), CA90097 (W. Rom), and ES00260. We thank Dr. Kazuhiko Ito for statistical analysis.

Abbreviations

PM	particulate matter
BPDE	benzo(a)pyrene diol epoxide
PAH	polycyclic aromatic hydrocarbon
NER	nucleotide excision repair
HCR	host cells reactivation
DTT	dithiothreitol
UV	ultraviolet
BPDE	benzo(a)pyrene diol epoxide
ROS	reactive oxygen species
TS	tobacco smoke
8-oxo-dG	8-oxo-deoxygunaine
LPO	lipid peroxidation
XP	xeroderma pigmentosum
NIST	National Institute of Standards and Technology
TSP	total suspended particles
F/U	fine/ultrafine
DMEM	Dulbecco's modified eagle's medium
PBS	phosphate buffered saline
MOUDI	micro-orifice uniform deposit impactor
TE	tris-EDTA buffer
HCR	host cell reactivation

REFERENCES

[1]. Alfaro-Moreno E, Nawrot TS, Nemmar A, Nemery B. Particulate matter in the environment: pulmonary and cardiovascular effects. Curr Opin Pulm Med. 2007; 13:98–106. [PubMed: 17255799]

[2]. Pope CA 3rd, Dockery DW. Health effects of fine particulate air pollution: lines that connect. J Air Waste Manag Assoc. 2006; 56:709–742. [PubMed: 16805397]

[3]. Nadadur SS, Kodavanti UP. Altered gene expression profiles of rat lung in response to an emission particulate and its metal constituents. Journal Of Toxicology And Environmental Health-Part A. 2002; 65:1333–1350. [PubMed: 12227955]

[4]. USEPA Air Quality Criteria for Particulate Matter. Vol. Vols I–II. US EPA, US Environmental Protection Agency; Washington DC: 2004.

[5]. Gutierrez-Castillo ME, Roubicek DA, Cebrian-Garcia ME, Vizcaya-Ruiz AD, Sordo-Cedeno M, Ostrosky-Wegman P. Effect of chemical composition on the induction of DNA damage by urban airborne particulate matter. Environmental And Molecular Mutagenesis. 2006; 47:199–211. [PubMed: 16355389]

[6]. Pope CA, Burnett RT, Thurston GD, Thun MJ, Calle EE, Krewski D, Godleski JJ. Cardiovascular mortality and long-term exposure to particulate air pollution - Epidemiological evidence of general pathophysiological pathways of disease. Circulation. 2004; 109:71–77. [PubMed: 14676145]

[7]. Feng ZH, Hu WW, Hu Y, Tang MS. Acrolein is a major cigarette-related lung cancer agent: Preferential binding at p53 mutational hotspots and inhibition of DNA repair. Proceedings Of The National Academy Of Sciences Of The United States Of America. 2006; 103:15404–15409. [PubMed: 17030796]

[8]. Kriek E, Van Schooten FJ, Hillebrand MJ, Van Leeuwen FE, Den Engelse L, De Looff AJ, Dijkmans AP. DNA adducts as a measure of lung cancer risk in humans exposed to polycyclic aromatic hydrocarbons. Environ Health Perspect. 1993; 99:71–75. [PubMed: 8319662]

[9]. Perera FP, Dickey C, Santella R, O'Neill JP, Albertini RJ, Ottman R, Tsai WY, Mooney LA, Savela K, Hemminki K. Carcinogen-DNA adducts and gene mutation in foundry workers with low-level exposure to polycyclic aromatic hydrocarbons. Carcinogenesis. 1994; 15:2905–2910. [PubMed: 8001254]

[10]. Smith LE, Denissenko MF, Bennett WP, Li H, Amin S, Tang M, Pfeifer GP. Targeting of lung cancer mutational hotspots by polycyclic aromatic hydrocarbons. J Natl Cancer Inst. 2000; 92:803–811. [PubMed: 10814675]

[11]. Hu WW, Feng ZH, Tang MS. Nickel (II) enhances benzo[a]pyrene diol epoxide-induced mutagenesis through inhibition of nucleotide excision repair in human cells: a possible mechanism for nickel (II)-induced carcinogenesis. Carcinogenesis. 2004a; 25:455–462. [PubMed: 14604891]

[12]. Risom L, Moller P, Loft S. Oxidative stress-induced DNA damage by particulate air pollution. Mutation Research-Fundamental And Molecular Mechanisms Of Mutagenesis. 2005; 592:119–137. [PubMed: 16085126]

[13]. Sorensen M, Autrup H, Hertel O, Wallin H, Knudsen LE, Loft S. Personal exposure to PM2.5 and biomarkers of DNA damage. Cancer Epidemiol Biomarkers Prev. 2003; 12:191–196. [PubMed: 12646506]

[14]. 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; 18:185–192. [PubMed: 9054605]

[15]. Reymao MS, Cury PM, Lichtenfels AJ, Lemos M, Battlehner CN, Conceicao GM, Capelozzi VL, Montes GS, Junior MF, Martins MA, Bohm GM, Saldiva PH. Urban air pollution enhances the formation of urethane-induced lung tumors in mice. Environ Res. 1997; 74:150–158. [PubMed: 9339228]

[16]. Esterbauer H, Schaur RJ, Zollner H. Chemistry And Biochemistry Of 4-Hydroxynonenal, Malonaldehyde And Related Aldehydes. Free Radical Biology And Medicine. 1991; 11:81–128. [PubMed: 1937131]

[17]. Hu WW, Feng ZH, Tang MS. Chromium(VI) enhances (+/−)-anti-7 beta,8 alpha-dihydroxy-9 alpha, 10 alpha-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-induced cytotoxicity and mutagenicity in mammalian cells through its inhibitory effect on nucleotide excision repair. Biochemistry. 2004b; 43:14282–14289. [PubMed: 15518579]

[18]. Feng ZH, Hu WW, Marnett LJ, Tang MS. Malondialdehyde, a major endogenous lipid peroxidation product, sensitizes human cells to UV- and BPDE-induced killing and mutagenesis through inhibition of nucleotide excision repair. Mutation Research-Fundamental And Molecular Mechanisms Of Mutagenesis. 2006; 601:125–136. [PubMed: 16872641]

[19]. Sancar A. DNA excision repair. Annual Review Of Biochemistry. 1996; 65:43–81.

[20]. Lehmann AR. The xeroderma pigmentosum group D (XPD) gene: one gene, two functions, three diseases. Genes & Development. 2001; 15:15–23. [PubMed: 11156600]

[21]. Gilmour MI, McGee J, Duvall RM, Dailey L, Daniels M, Boykin E, Cho SH, Doerfler D, Gordon T, Devlin RB. Comparative toxicity of size-fractionated airborne particulate matter obtained from different cities in the United States. Inhal Toxicol. 2007; 19(Suppl 1):7–16. [PubMed: 17886044]

[22]. Liu W, Zhang J, Kwon J, Weisel C, Yurpin B, Zhang L, Korn L, Morandi M, Stock T, Colome S. Concentrations and source characterics of airborne carbonyl compounds measured outside urban residences. J. Air & Waste Manage Assoc. 2006; 56:1196–1204.

[23]. Reibman J, Hsu Y, Chen LC, Kumar A, Su W, Choy W, Talbot A, Gordon T. Size-fractions of ambient particulate matter induce granulocyte-macrophage colony stimulating factor in human bronchial epithelial cells by mutagen activated protein kinase pathways. Am. J. Respir. Cell. Molec. Biol. 2002; 27:455–462. [PubMed: 12356579]

[24]. Jia L, Wang XW, Harris CC. Hepatitis B virus X protein inhibits nucleotide excision repair. International Journal Of Cancer. 1999; 80:875–879.

[25]. Wood RD, Robins P, Lindahl T. Complementation Of The Xeroderma Pigmentosum Dna-Repair Defect In Cell-Free-Extracts. Cell. 1988; 53:97–106. [PubMed: 3349527]

[26]. Wang Z, Wu X, Friedberg EC. Methods in Enzymology. 1995; 7 7:177–186.

[27]. Lee DH, O'Connor TR, Pfeifer GP. Oxidative DNA damage induced by copper and hydrogen peroxide promotes CG -> TT tandem mutations at methylated CpG dinucleotides in nucleotide excision repair-deficient cells. Nucleic Acids Research. 2002; 30:3566–3573. [PubMed: 12177298]

[28]. Feng ZH, Hu WW, Amin S, Tang MS. Mutational spectrum and genotoxicity of the major lipid peroxidation product, trans-4-hydroxy-2-nonenal, induced DNA adducts in nucleotide excision repair-proficient and -deficient human cells. Biochemistry. 2003; 42:7848–7854. [PubMed: 12820894]

[29]. Pope CA, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K.e.a. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. Jama-Journal Of The American Medical Association. 2002; 287:1132–1141.

[30]. Friedberg EC. How nucleotide excision repair protects against cancer. Nat Rev Cancer. 2001; 1:22–33. [PubMed: 11900249]

[31]. Feng ZH, Hu WW, Tang MS. Trans-4-hydroxy-2-nonenal inhibits nucleotide excision repair in human cells: A possible mechanism for lipid peroxidation-induced carcinogenesis. Proceedings Of The National Academy Of Sciences Of The United States Of America. 2004; 101:8598–8602. [PubMed: 15187227]

[32]. Friedburg, EC.; Walker, GC.; Siede, W.; Wood, RD.; Schulz, RA.; Ellenberger, T. DNA Repair and Mutagenesis. ASM Press; Washington, D.C: 2006. Mechanism of nucleotide excision repair in eukaryotes; p. 317-350.

[33]. Bal W, Schwerdtle T, Hartwig A. Mechanism of nickel assault on the zinc finger of DNA repair protein XPA. Chem Res Toxicol. 2003; 16:242–248. [PubMed: 12588196]

[34]. Kopera E, Schwerdtle T, Hartwig A, Bal W. Co(II) and Cd(II) substitute for Zn(II) in the zinc finger derived from the DNA repair protein XPA, demonstrating a variety of potential mechanisms of toxicity. Chem Res Toxicol. 2004; 17:1452–1458. [PubMed: 15540943]

[35]. Mackay JP, Crossley M. Zinc fingers are sticking together. Trends In Biochemical Sciences. 1998; 23:1–4. [PubMed: 9478126]

[36]. Moolenaar GF, Moorman C, Goosen N. Role of the Escherichia coli nucleotide excision repair proteins in DNA replication. Journal Of Bacteriology. 2000; 182:5706–5714. [PubMed: 11004168]

[37]. Hartwig A. Recent advances in metal carcinogenicity. Pure And Applied Chemistry. 2000; 72:1007–1014.

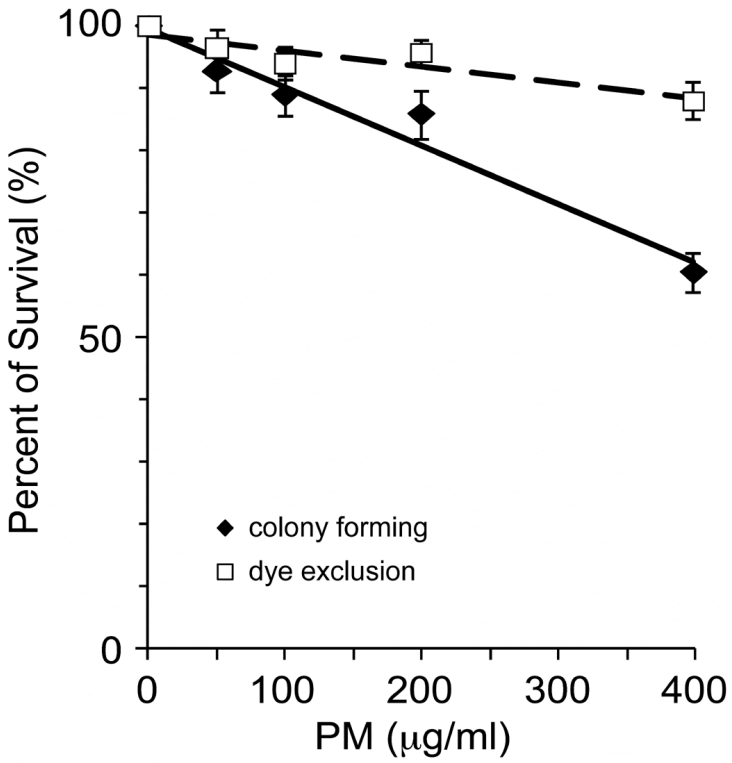

Figure 1. Effect of fine/ultra-fine PM on cell viability

Logarithmically growing A549 cells were treated with F/U PM for 24 h, trypsinized, and the viability of treated cells was detected by Trypan Blue dye exclusion assay or by colony forming ability assay. The data represents three independent experiments. The bar represents range of the experimental results and the position of the symbol represents the average value. TSP treatment induced a similar effect on cell viability (Trypan Blue dye exclusion assay, data not shown).

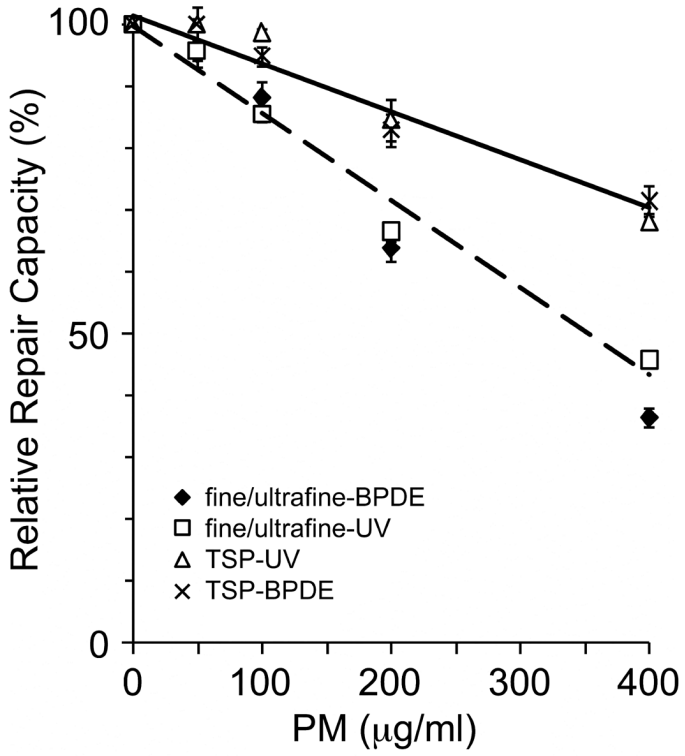

Figure 2. Effects of PM on repair of bulky DNA damage determined by host cell reactivation (HCR) assay

Logarithmically growing A549 cells were treated with different concentrations of PM (0–400 μg/ml) for 24 h. PM was removed and the cells were transfected with UV or BPDE modified-irradiated pGL3-luciferase and unmodified pSV-β-galactosidase plasmids using Fugene 6 for 16 h. The cells were further incubated with the same concentrations of PM in growth medium for 24 h. Cell lysates were then prepared and the luciferase and β-galactosidase activity was measured. The relative repair capacity of cells was calculated as the percentage of the relative luciferase activity of the plasmids transfected into PM treated cells as compared to untreated cells. The bar represents range of the experimental results and the position of the symbol represents the average value.

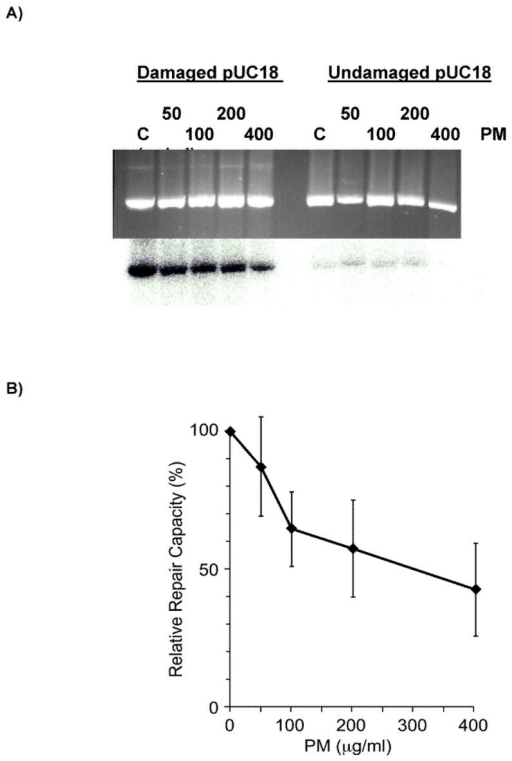

Figure 3. Effects of fine/ultra-fine PM treatment on the cell capacity in mediating DNA-damage induced repair synthesis

Logarithmically growing A549 cells were treated with different concentrations of PM (0–400 μg/ml) for 24 h. Cell lysates were prepared immediately after **F/U** PM treatment and used to mediate repair synthesis in UV-damaged pUC18 plasmid and undamaged control pUC18 plasmid in the presence of α-^{32}P dATP. Plasmid DNAs were then purified, digested with HindIII, and separated in a 1 % agarose gel by electrophoresis. A) The upper panel is a typical photograph of ethidium bromide-stained gel and the lower panel is an autoradiograph of the same gel. B) The relative repair capacity was calculated based on the ratio of amount of repair synthesis over the amount of substrate DNA. The data represent three independent experiments. The bar represents range of the experimental results and the position of the symbol represents the average value.

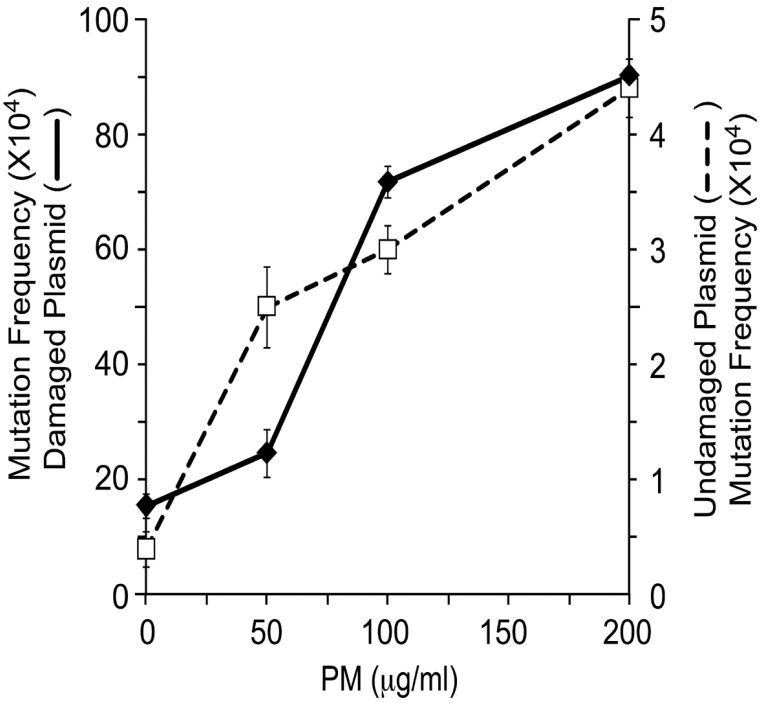

Figure 4. PM exposure enhances both spontaneous and UV-induced mutagenesis

Methods for F/U PM exposure, transfection and *supF* gene mutation detection are described in Materials and Methods. The mutant plasmids were purified and the mutations in *supF* gene in these plasmids were confirmed by DNA sequencing. Mutation frequency was calculated based on the ratio of the number of white colony over the total colony number. The bar represents range of the experimental results and the position of the symbol represents the average value.

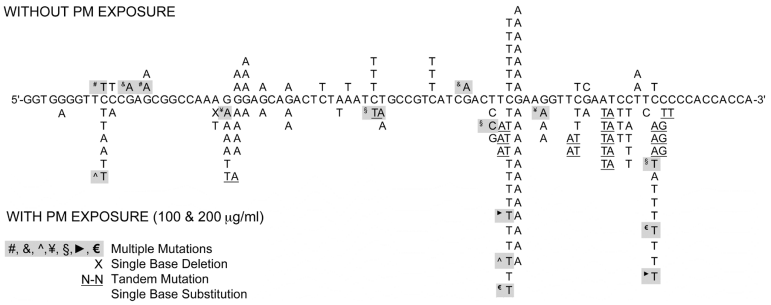

Figure 5. Effect of fine/ultra-fine PM exposure on UV-induced mutational spectrum in the *supF* gene in pSP189 plasmids replicated in human lung cells (A549) with (lower panel) or without (upper panel) PM exposure

Methods for F/U PM exposure, transfection and *supF* gene mutation detection are described in Materials and Methods. The mutant plasmids were purified (86 mutant plasmids from PM exposed cells and 37 mutants plasmids from cells without PM exposure) and the *supF* gene in these plasmids was sequenced. The types of single base substitutions are shown above and below the *supF* sequence. The other symbols denoted are as follows: X, single base deletions; #, &, ^, ¥, §, ▶ and €, multiple mutations (more than one mutation occurring in the same plasmid); N-N, tandem mutations (mutations occur at two adjacent bases).

Table I

Types of mutations in the *supF* gene in UV-damaged pSP189 plasmid replicated in A549 cells treated with fine/ultra-fine PM.

Type of Mutation	Control	Treated
Single base substitution	35 (94.6%)	64 (74.4%)
Single base deletion	0 (0%)	1 (1.1%)
Tandem mutation	0 (0%)	16 (18.6%)
Double mutation	2 (5.4%)	5 (5.8%)