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DNA damage response of A549 cells treated with particulate matter (PM_{10}) of urban air pollutants

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ABSTRACT

We describe the events triggered by a sub-lethal concentration of airborne particulate matter (PM_{10}) in A549 cells, which include the formation DNA double-strand breaks, γH2A.X generation, and 53BP1 recruitment. To protect the genome, cells activated ATM/ATR/Chk1/Chk2/p53 pathway but, after 48 h, cells turned into a senescence-like state. Trolox, an antioxidant, was able to prevent most of the alterations observed after particulate matter exposure, demonstrating the important role of ROS as mediator of PM_{10}-induced genotoxicity and suggesting that DNA damage could be the mechanisms by which particulate matter augment the risk of lung cancer.

1. Introduction

There is growing concern that exposure to airborne particulate matter (PM) is associated with an increase in morbidity and mortality and that it could augment the risk of lung cancer [1–3]. PM of an aerodynamic diameter $\leqslant$10 mm (PM_{10}) consists of dust, soot, and other solid, liquid, and aerosol particles, as well as its chemical constituents. Although the mechanisms of PM_{10}-related health effects remain incompletely studied, there is increasing evidence that exposure to PM_{10} has a major effect in several experimental models [4,5]. In addition, some of these changes are strongly related to an increase in reactive oxygen species (ROS) [6,7]. In this regard, one of the most important changes induced by PM_{10} is DNA damage [8,9] and the level of this damage has been correlated with mutagenicity and tumor formation in experimental models [10,11]. The induced DNA damage by PM_{10} and its constituents involves the formation of DNA double-strand breaks (DSBs) [8,9,12,13]. In response to DSBs caused by other agents like ionizing radiation [14], the histone H2A.X undergoes phosphorylation at Ser139 [14]. The phosphorylated H2A.X protein is defined as γH2A.X and this phosphorylation is mediated by phosphoinositide 3-kinase-related protein kinases (PIKKs), including ataxia telangiectasia mutated (ATM), ATM and Rad-3 related kinase (ATR), and DNA dependent protein kinase (DNA-PKcs) [15]. ATM and ATR, in turn, activate the checkpoint kinases, Chk1 and Chk2, and tumor suppressor 53-binding protein 1 (53BP1). 53BP1 binds to the DNA-binding domain of p53, enhances p53-mediated transcriptional activation, and rapidly colocalizes with γH2A.X in response to DNA damage induced by ionizing radiation [16]. In addition, Chk1 and Chk2 kinases have several effectors, including cell division 25 (Cdc25) family proteins, which have phosphatase activity related to cell cycle regulation, specifically Cdc25A, in response to ionizing radiation [17]. On the other hand, after DNA damage,

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p53 initiates different transcriptional programs that lead to cell cycle arrest, cellular senescence, or apoptosis in response to γ-radiation through the ATM/ATR and Chk1/Chk2 kinases [18]. To this regard, before p53 induction, a disruption between the complex formed by p53 and MDM2, a p53-specific E3 ubiquitin ligase, is needed for its biological response [19]. In the setting of DNA damage, the cell undergoes cell cycle arrest to provide time to carry out DNA repair [9,13] or apoptosis, depending on the PM_{10} concentration used. Based on this information, we decided to investigate if the above cell-signaling pathway (ATM/ATR/Chk1/Chk2/p53) is involved in the response to the DNA damage induced by PM_{10} exposure in epithelial lung cancer cells even when cells are exposed to a sub-lethal concentration. Since there is an increase in DNA damage correlated to the oxidative stress induced by environmental pollutants [20,21], we decided to use trolox, a hydroxyl radical scavenger, to investigate if a connection exists between the effect of PM_{10} exposure on DNA damage and if this effect is related to ROS formation. This study evidences that PM_{10} activates sensors (53BP1), transducers (ATM/ATR), and effectors (H2A.X, Chk1, Chk2, p53, and MDM2) of DNA damage, pointing toward a strong relation between DNA damage and oxidative stress. Even though, this signal transduction pathway is activated in response to DNA damage after 24 h, a senescence-like phenotype is observed in cells exposed to PM_{10} after 48 h. These findings provide important clues on the role of PM_{10} exposure in DNA damage and senescence-like events that are strongly involved in cancer development.

2. Materials and methods

2.1. PM_{10} sampling

PM_{10} was collected in a commercial zone (with major traffic sources) of Mexico City using a high-volume particle collector with a flux of 1.13 m^3/min (GMW model 1200 VFC HVPM10; Sierra Andersen, Smyrna, GA, USA). PM_{10} was collected on 3.0-μm pore size cellulose nitrate filters (Sartorius AG, Goettingen, Germany), 3 days a week from October 2004–May 2005. Filters were maintained in the dark at 4 °C in a desiccator prior to particle removal. Particles were gently scraped off the membranes with a surgical blade into endotoxin-free glass vials, maintained in the dark in a dryer at 4 °C until use.

2.2. Cell culture and PM_{10}/trolox treatment

A549 type cells derived from human adenocarcinoma were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in F12 K medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum in a 5% CO_2 atmosphere at 37 °C. Cells were seeded in 6-well culture plates (1×10^6 cells per well) and they were grown in F12K medium supplemented with 10% fetal bovine serum for 24 h. Then the medium was removed and replaced to free serum medium plus PM_{10}. Free serum medium was used in order to avoid that albumin contained in the fetal bovine serum acts as metal chelator and masks the real PM_{10} effect because it contains metals [22]. Since PM has a complex composition that includes soluble and insoluble compounds and it was not totally dissolved in culture medium, a suspension of PM_{10} was obtained and the A549 cell culture was exposed to this suspension at 10 μg/cm^2 during 24 h. Parallel experiments were done after pre-incubation with 10 μM trolox (antioxidant).

2.3. The comet assay

A549 cells were plated on 24-well plates (2×10^4 cells per well) and exposed to 10 μg/cm^2 of PM_{10} and, after 24 h of exposure, cells were trypsinized (0.05%). Cells (1×10^5) were mixed in 1% low fusion point agarose and then placed on a slide covered with previously solidified agarose. The sample was treated with digestion buffer (10 mM Tris, 100 mM EDTA, 2 M NaCl, 1% Triton X-100, pH 10) for 30 min, then rinsed in a 100 mM NaCl, 1 mM EDTA solution, and subjected to 50 V alkaline electrophoresis for 30 min (Horizon 58; GIBCO-BRL, Gaithersburg, MD). SYBER Green I solution (TREVIGEN cat.4250-050-K) was added to each sample and analyzed under fluorescence microscopy (AX70; Olympus, Japan), capturing the image with a digital camera (Coolpix P5100, Nikon). The length of each comet was measured using an image analysis program (ImageJ, NIH) [23]. One hundred comets per sample were measured. Length distributions were used and the median, 25th and 75th percentiles, and maximal and minimal values were calculated.

2.4. Western blot

The cells were rinsed twice with PBS and lysed with 20 mM Tris, 1% NP-40, and 150 mM NaCl, pH 8.0; a protease inhibitor cocktail (Roche Mannheim, Germany) and the cell lysates were centrifuged (13000 *g* for 2 min), and the supernatant was stored at −20 °C.

The protein content of the supernatant was calculated using the bicinchoninic acid protein assay [24]. SDS polyacrilamide gels were prepared by standard techniques and 40 μg of protein was loaded per line onto a 12.5% acrylamide-SDS gel [25]. Protein was blotted onto a PVDF membrane (Immobilon-P, Millipore, UK) and probed using primary and secondary antibodies and detected by enhanced chemiluminescence (Millipore, UK), following the Towbin et al. procedure [26]. H2A.X (07146), phosphorylated H2A.X (γH2A.X) (07164), ATM (05513), phospho-ATM S1981 (05740) were purchased from Upstate; ATR (ab2905), phospho-ATR S428 (2853), Chk1 (ab47488), phospho-Chk1 S317 (ab38518), Chk2 (ab47433), phospho-Chk2 T68 (E126), 53BP1 (ab21083), MDM2 (ab53710), Cdc25A (2357), phospho-Cdc25A S278 (ab59977) were purchased from Abcam (Cambridge, MA). Actin antibody was utilized as loading control (kindly provided by Dr. Manuel Hernández (Department of Cellular Biology, CINVESTAV-IPN, Mexico City, Mexico). Equal protein concentration loading on the PVDF membrane was verified using Ponceau S staining (Sigma, USA). Protein levels were evaluated through densitometry using the Image J freeware program (rsb.info.nih.gov/ij) [27].