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Integrative transcriptomic and protein analysis of human bronchial BEAS-2B exposed to seasonal urban particulate matter

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ABSTRACT

Background: Exposure to particulate matter (PM) is associated with various health effects. Physico-chemical properties influence the toxicological impact of PM, nonetheless the mechanisms underlying PM-induced effects are not completely understood.
Objectives: Human bronchial epithelial cells were used to analyse the pathways activated after exposure to summer and winter urban PM and to identify possible markers of exposure.
Methods: BEAS-2B cells were exposed for 24 h to 10 μg/cm^2 of winter PM2.5 (wPM) and summer PM10 (sPM) sampled in Milan. A microarray technology was used to profile the cells gene expression. Genes and microRNAs were analyzed by bioinformatics technique to identify pathways involved in cellular responses. Selected genes and pathways were validated at protein level (western blot, membrane protein arrays and ELISA).
Results: The molecular networks activated by the two PM evidenced a correlation among oxidative stress, inflammation and DNA damage responses. sPM induced the release of pro-inflammatory mediators, although miR-146a and genes related to inflammation resulted up-regulated by both PM. Moreover both PM affected a set of genes, proteins and miRNAs related to antioxidant responses, cancer development, extracellular matrix remodeling and cytoskeleton organization, while miR-29c, implicated in epigenetic modification, resulted up-regulated only by wPM. sPM effects may be related to biological and inorganic components, while wPM apparently related to the high content of organic compounds.
Conclusions: These results may be helpful for the individuation of biomarkers for PM exposure, linked to the specific PM physico-chemical properties.

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1. Introduction

Particulate matter (PM) is nowadays considered one of the major environmental risk to health in the world, responsible for 3.7 million annual premature deaths (WHO, 2014) for induction of pulmonary and cardiovascular diseases, and lung cancer (Loomis et al., 2013; Kim et al., 2015). Despite the international research effort, several aspects concerning the biological mechanisms responsible for PM-induced health effects remain unclear. Recent studies have underlined the role of PM physico-chemical characteristics in promoting its specific biological outcomes. Moreover several reports (Cassee et al., 2013; Kim et al., 2015; Rohr and Wyzga, 2012) suggest that the presence of certain PM components may be more significant than PM concentration itself in inducing health effects. Nevertheless researchers are far from having a detailed mechanistic explanation of the causal relation between PM and health effects suggested by epidemiological

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evidences.

Toxicological studies on *in vitro* cell systems are crucial to investigate the cellular mechanisms and provide support to clinical and epidemiological observation (Huang, 2013). Oxidative stress, inflammation and DNA damage are the main PM-induced cell outcomes (Nemmar et al., 2013). All these processes have an important role in eliciting asthma, allergy, chronic obstructive pulmonary disease (COPD), cardiovascular diseases and cancer, however the molecular pathways involved can be several and related to different biological mechanisms.

Accumulating evidences (Nel, 2005; Fahmy et al., 2010) demonstrate that fine PM, namely particles with mean aerodynamic diameter lower than 2.5 microns ($PM_{2.5}$), mainly composed of combustion-derived particles with a high redox capacity, polycyclic aromatic hydrocarbons (PAHs) and metals, is responsible for the different health effects. Epidemiological data report that the exposure to vehicular emissions is associated to cardiovascular mortality and morbidity, asthma exacerbation and other respiratory diseases (Rohr and Wyzga, 2012). These results are confirmed by toxicological studies on diesel emissions, diesel exhaust particles (DEP), and particulate samples collected near highways in high trafficked cities, demonstrating that fine PM induces biological responses related to health diseases (Ghio et al., 2012; Grahame and Schlesinger, 2010). The importance of the organic fraction adsorbed on fine PM in determining cell damages has been extensively analysed and significant activation of signalling pathways and gene expression reported (Andrysík et al., 2011; Líbalová et al., 2014).

Besides, a positive associations between coarse PM (PM10–2.5) and respiratory and cardiovascular diseases and mortality has been evidenced by epidemiological data (Cassee et al., 2013; Huang et al., 2011; Kelly and Fussell, 2012). Coarse PM is dominated by mechanically abraded particles, minerals, metals and biological components, which play a central role in health effects, stimulating the alveolar macrophages and the respiratory cells to release pro-inflammatory cytokines and chemokines (Nemmar et al., 2013). It has also been reported that biological compounds may act together with other PM components, such as diesel exhaust, to enhance IgE production and promoting allergic sensitization (Schwarze et al., 2010). Moreover the presence of transition metals with a strong redox activity, such as vanadium and nickel (residual oil fly ash), iron, manganese and molybdenum (engine abrasion), and copper or antimony (brake wear), determines an increment of PM10 oxidative potential (Cassee et al., 2013).

The complex PM's chemical composition is responsible for our lack of understanding of the causal relation between particles inhalation and human diseases; due to this complexity, the possibility to uncover the mechanisms of action require the use of high throughput approaches in support of classical toxicological analyses.

During these last years "omics" approaches have been used to investigate complex molecular mechanisms. Toxicogenomics, including in also transcriptomics, has demonstrated its utility to assess the toxicity of drugs on human cell lines and to clarify the action mechanism of new compounds (Hartung, 2009; Jennings et al., 2013). Global gene expression profiling has been increasingly used to investigate PM-associated health effects; gene modulation was found in pathways related to oxidative stress, metabolism of xenobiotics, inflammatory cytokines and Toll-like receptor signalling (Huang, 2013), supporting the toxicological evidences of PM biological effects. Other pathways related to i) cell cycle regulation, ii) apoptosis, iii) mitogen-activated protein kinase (MAPK) signaling, iv) T- and B-cell receptor signaling, v) metal binding, vi) cell aging and vii) cancer development, resulted modulated in relation to the particles chemical composition. Moreover the changes in miRNA expression have been suggested to be a novel mechanism involved in PM response (Bollati et al., 2010) and miRNAs have been proposed as novel indicators of environment exposure (Vrijens et al., 2015). Therefore the transcriptomic approach, integrating gene and miRNA analysis, can be useful to find the biological linkage between PM health effects and the promoting compounds.

The present study intends to evaluate the signaling pathways associated to winter PM2.5 (wPM) and summer PM10 (sPM), on human bronchial BEAS-2B cells by means of a global transcriptomic profiling and to investigate selected pathways at protein level. MiRNAs have also been analyzed to identify their role in PM-induced effects and to evaluate their potential as markers of exposure.

2. Material and methods

2.1. PM collection and preparation

Summer PM10 (sPM) and winter PM2.5 (wPM) samples were collected at Torre Sarca (Milan), a representative urban site. Sampling and detaching of particles from filters were performed as previously reported (Gualtieri et al., 2012). The mean particles recovery in the present study was of 75%, as determined by the ratio of mass of particles collected after extraction over the mass of particles sampled on filters.

2.2. Cell culture and treatments

The human bronchial epithelial BEAS-2B cells (European Collection of Cell Cultures, ECACC, Salisbury, UK) were maintained in LCH-9 medium (Gibco-Invitrogen) and plated on collagen (PureColTM)-coated flasks. Cells were maintained at 37 °C in humidified atmosphere with 5% CO_2; the medium was replaced every two days. Cells were seeded into 6-well culture plates at 80,000 cells/well and treated after 48 h with 10 $\mu g/cm^2$ of summer PM10 or winter PM2.5 for 24 h.

2.3. RNA extraction

Cells were lysed and stored in QIAzol Lysis reagent (Qiagen, Hilden, Germany) until the RNA extraction. Total RNA was extracted from cells using the miRNeasy extraction kit (Qiagen, Hilden, Germany) and eluted in RNase free-water, according to the manufacturer's recommended guidelines. RNA samples quantification was performed using ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and the quality was checked with 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). Only RNA samples with RIN (RNA Integrity Number) $\geq$ 9 and presence of low molecular weight RNA molecules (including 5S rRNA and small RNAs) were considered for the experiments. 1 μg of total RNA was used for gene and microRNA expression profiling.

2.4. Global gene expression analysis by GeneChip

Gene expression were analysed in the human bronchial epithelial cell line, BEAS-2B exposed to wPM and sPM trough microarray analysis. Unexposed cells were used as control and the experiment was performed in triplicate. Biotin-labelled target was prepared from total RNA according to GeneChip® Expression Analysis Technical Manual protocol (Affymetrix, Santa Clara, CA, USA). Samples were fragmented and 15 μg of the obtained cRNA were hybridized at 45 C° for 16 h onto GeneChip® Human U133 Plus 2, nonspecifically bound material was removed by washing. Specifically bound targets were detected using GeneChip®

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