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Genotoxic and epigenotoxic effects of fine particulate matter from rural and urban sites in Lebanon on human bronchial epithelial cells

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ABSTRACT

Assessment of air pollution by particulate matter (PM) is strongly required in Lebanon in the absence of an air quality law including updated air quality standards. Using two different $PM_{2.5–0.3}$ samples collected at an urban and a rural site, we examined genotoxic/epigenotoxic effects of PM exposure within a human bronchial epithelial cell line (BEAS-2B). Inorganic and organic contents evidence the major contribution of traffic and generating sets in the $PM_{2.5–0.3}$ composition. Urban $PM_{2.5–0.3}$ sample increased the phosphorylation of H2AX, the telomerase activity and the miR-21 up-regulation in BEAS-2B cells in a dose-dependent manner. Furthermore, urban $PM_{2.5–0.3}$ induced a significant increase in *CYP1A1*, *CYP1B1* and AhRR genes expression. The variable concentrations of transition metals and organic compounds detected in the collected $PM_{2.5–0.3}$ samples might be the active agents leading to a cumulative DNA damage, critical for carcinogenesis.

1. Introduction

In October 2013, the International Agency for Research on Cancer (IARC) classified outdoor air pollution and fine particulate matter (PM) as *carcinogenic to humans* (Group 1) (Loomis et al., 2013). Several epidemiological and toxicological studies have documented the remarkable effect of PM with aerodynamic diameter ≤ 2.5 μm ($PM_{2.5}$) in increasing morbidity and mortality in patients that suffer from respiratory diseases and lung cancer (Longhin et al., 2013a, 2013b; Pope et al., 2011) and in causing inflammatory reactions and oxidative stress in lung cells exposed to these particles (Cachon et al., 2014; Dergham et al., 2012; Dieme et al., 2012).

In Beirut (capital of Lebanon) as many cities in the world, air quality assessment data reported that the air $PM_{2.5}$ concentrations currently exceed air quality international standards and guidelines (Massoud et al., 2011; Waked and Afif, 2012; Waked et al., 2012). To date, very few studies have been conducted in Lebanon to assess air pollution exposure and the potential risk for diseases development (Borgie et al., 2014; Kobrossi et al., 2002; Mrad Nakhle et al., 2013; Salameh et al., 2012). Although the majority of published studies have defined the origin and the variability of particulate matter (PM_{10} and $PM_{2.5}$) mass concentrations over Beirut area, their adverse health effects using $PM_{2.5}$ experimental exposure studies depending upon its natural and/or anthropogenic emission source are usually not well established. To investigate the association between PM composition, collected from different

Abbreviations: AhR, aryl hydrocarbon receptor; AhRR, aryl hydrocarbon receptor repressor; ARNT, AhR nuclear translocator; B[a]P, Benzo[a]Pyren; Ct, total carbon; CYP1A1, cytochrome P4501A1; CYP1B1, cytochrome P4501B1; s, double strand breaks; DL, detection limit; LDH, lactate dehydrogenase; MDH, mitochondrial dehydrogenase; miRNA, microRNA; NQO1, Nicotinamide adenine dinucleotide phosphate quinoneoxydoreductase 1; PAHs, PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzo-p-dioxins; T, tetra; pe, penta; Hx, hexa; Hp, hepta; O, octa-PCDDs; PCDFs, polychlorinated dibenzofurans; PM, particulate matter; R, rural; ROS, reactive oxygen species; U, urban; XRE, xenobiotics responsive elements

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sites, with particles genotoxic and epigenotoxic effects on cells, detailed *in vitro* studies are highly needed (Boogaard et al., 2012; Lippmann et al., 2013).

Air PM fine fraction is a heterogeneous mixture of various types of particles originating from natural and anthropogenic sources. Indeed, PM-induced biological effects changed with the origin of the particles, and such variations may be explained by the difference between the chemical constituents of PM according to the studied sites (Perrone et al., 2013). Thus, oxidation–reduction of metal ions in PM (*e.g.*, iron and copper) has been shown to generate reactive oxygen species (ROS) *via* the Fenton reaction (Boogaard et al., 2012; Janssen et al., 2014). Moreover, polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) have a well-known genotoxic potential (Hung et al., 2013; Shiizaki et al., 2013).

Air pollution fine particles-related genotoxicity is well discussed (Abbas et al., 2013; Andre et al., 2011; Jardim et al., 2009; Lepers et al., 2014; Longhin et al., 2013a). However, the underlying mechanisms by which PM-induced genotoxicity in lung cells can cause adverse health effects are still not totally clear, especially PM-induced double strand DNA breaks (DSBs) (Gualtieri et al., 2011) and induction of telomerase activity (Büchner et al., 2013). Remarkably, the most severe DNA lesions produced by ROS are DSBs (Mills et al., 2003) that are detected by the phosphorylated histone H2AX (γH2AX) (Albino et al., 2009). Interestingly, telomerase is required for the addition of telomeric repeats to the ends of linear chromosomes, and thus, it is generally activated in lung cancer (Fiorito et al., 2014; Pereira and Ferreira, 2013).

On the other hand, the recently discovered role of epigenetic modifications in tumors has been also proven in lung carcinogenesis (Luzhna et al., 2013). Many recent studies have associated air pollution PM to epigenetic alterations, especially DNA methylation and histone modifications (Baccarelli et al., 2009; Cantone et al., 2011; Madrigano et al., 2011) but more studies are needed in this field. Contrariwise, little is known about the effect of PM air pollution on microRNAs (miRNAs; small noncoding RNAs) expression (Bollati et al., 2010; Jardim et al., 2009). Indeed, miRNAs have been suggested to be important in maintaining the lung in a disease-free state through regulation of gene expression (Fossati et al., 2014). Moreover, it has been estimated that half of them are epigenetically regulated, while epigenetic machinery is also targeted by miRNAs, demonstrating how these gene regulatory pathways are involved in tumorigenesis (Motta et al., 2013). Of note, changes in the expression of miR-21 have been implicated in disease mechanisms that may be related to PM exposure, such as oxidative stress (Bollati et al., 2010). In addition, it has been demonstrated that the expression of miR-21 is upregulated during the course of the inflammatory response (Iliopoulos et al., 2010; Lu et al., 2009; Luo et al., 2013; Sen and Roy, 2012; Tili and Michaille, 2011). This miRNA is also considered oncogene due to its upregulation of expression in several types of cancers (Asangani et al., 2008; Corsten et al., 2007; Ribas et al., 2009; Wang et al., 2009) including lung cancer (Liu et al., 2011; Seike et al., 2009).

Moreover, PAHs require a metabolic activation by the cytochrome P450 1 (CYP1) superfamily members to produce the reactive intermediates eliciting their adverse health effects (Billet et al., 2008).

The aryl hydrocarbon receptor (AhR) and its DNA binding partner, the AhR nuclear translocator (ARNT) are central in the regulation of CYP enzymes and mediating PAH-induced gene expression. AhR repressor (AhRR) inhibits AhR signaling through a proposed mechanism involving competition with AhR for dimerization with ARNT and binding to xenobiotics responsive elements (XRE) (Haarmann-Stemmann and Abel, 2006). Recent studies have shown that competition between AhR and AhRR for binding to XRE or ARNT does not fully explain the repressor function of AHRR (Evans et al., 2008; Haarmann-Stemmann et al., 2007; Matthews, 2013) and further studies are needed to elucidate this mechanism.

In order to clarify the underlying molecular mechanisms involved in air pollution $PM_{2.5–0.3}$-induced human lung genotoxic and epigenotoxic events, two $PM_{2.5–0.3}$ samples were collected at urban and rural sites in Lebanon. First, we studied their most relevant physicochemical characteristics and then, we investigated *in vitro* toxicity of the two $PM_{2.5–0.3}$ samples on a human bronchial epithelial cell model (BEAS-2B cell line) by monitoring mitochondrial dehydrogenase (MDH) and extracellular lactate dehydrogenase (LDH) activities. We also examined whether exposure of BEAS-2B cells to both rural and urban PM resulted in genotoxic effects by the evaluation of phosphorylated H2AX and the telomerase activity. Furthermore, the ability of the two $PM_{2.5–0.3}$ samples to induce a gene regulation of three candidate miRNAs, miR-21, miR-26b and miR-27a in BEAS-2B cells was evaluated. Subtle changes in the expression of *AhR* and *ARNT*, as well as *AhR/ARNT*-regulated genes such as *AhRR*, *CYP1A1*, *CYP1B1* and *NQO1* were also investigated.

2. Materials and methods

2.1. PM sampling and characterization

PM collection. Fine particulate matter ($PM_{2.5–0.3}$) samples were collected in an urban background site (33°52'46.76"N; 35°32'14.53"E; Sin-El-Fil, called U) of Beirut (2 million inhabitants) influenced by vehicular traffic, and in a rural site located near Byblos (34°10'33.02"N; 35°42'58.48"E; Bejje, called R; 1560 inhabitants; 35 km north of Beirut). The first site is located in the city of Beirut and affected by the emission of various human activities, mainly traffic, in addition to natural sources. At Sin-El-Fil, the transit traffic is regular throughout the day and dense during peak hours: the fleet is characterized by the presence of private cars running on gasoline, buses and trucks running on diesel. Bejje is a rural site, where the car use is local and very light.

$PM_{2.5–0.3}$ particles sampling was performed using a high volume six stages cascade impactor Staplex® model 236 (TFIA-2, EU system 42 m^3/h, Mesurex, France) continuously during the Mediterranean climate dry season from May until October 2011. Impaction plates were mounted without any filter except on the first stage in order to retain particles with highest diameters. The impaction system was changed every 15 days in order to collect sufficient masses of $PM_{2.5–0.3}$ at both sites and therefore to be able to study both physicochemical characteristics and toxicological end points. After sampling, impaction plates were dried under laminar flow hood during 48 h, and then $PM_{2.5–0.3}$ were recovered from the collection plates using a brush and immediately stored at −20 °C. At both sites, the whole of 15 days particles samples were mixed carefully together and then stored at −20 °C until physicochemical and toxicological analysis. 500 mg and 224 mg of U and R $PM_{2.5–0.3}$ were obtained respectively. Meteorological data (*i.e.*, wind speed, wind direction, temperature and rain) were obtained from the general directorate of civil aviation of the Beirut international airport station network. During the sampling campaign, from May until October 2011, the recorded temperatures varied between 20 °C and 32 °C, rain falls were low (42 mm), and low speed winds were observed and mainly originated from the South to West quarter direction. All these data indicated that the climatic conditions during the studied period were in agreement with the historical meteorological data.

PM physicochemical characterization. Physicochemical characterization of the two $PM_{2.5–0.3}$ samples was carried out as published by Cazier et al. (2011) and Ledoux et al. (2006). Briefly,