Experimental Gerontology 110 (2018) 125–132

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Influence of aging in the modulation of epigenetic biomarkers of carcinogenesis after exposure to air pollution

Bertrand Fougère[a,b,*], Yann Landkocz[b], Capucine Lepers[b], Perrine J. Martin[b], Lucie Armand[b], Nicolas Grossin[c], Anthony Verdin[b], Eric Boulanger[c,e], Pierre Gosset[b,d], François Sichel[f], Pirouz Shirali[b], Sylvain Billet[b]

[a] GCS-Groupement des Hôpitaux de l'Institut Catholique de Lille, Gerontology Clinic, Faculté Libre de Médecine, Lille 59000, France
[b] Unité de Chimie Environnementale et Interactions sur le Vivant (EA4492), Université du Littoral Côte d'Opale, Dunkerque 59140, France
[c] University of Lille 2, Biology of Aging (EA2693), Lille 59000, France
[d] GCS-Groupement des Hôpitaux de l'Institut Catholique de Lille, Department of Anatomy and Pathology, Faculté Libre de Médecine, Lille 59000, France
[e] Gerontology Clinic, Les Bateliers Geriatric Hospital, Lille University Hospital, Lille 59000, France
[f] Normandie Univ, UNICAEN, ABTE EA4651, Centre de Lutte Contre le Cancer François Baclesse, Caen 14000, France

ARTICLE INFO

Section Editor: Werner Zwerschke

Keywords:
$PM_{2.5}$
PBMC
Biomarkers
Epigenotoxicity
Aging

ABSTRACT

Background: Classified as carcinogenic to humans by the IARC in 2013, fine air particulate matter ($PM_{2.5}$) can be inhaled and retained into the lung or reach the systemic circulation. This can cause or exacerbate numerous pathologies to which the elderly are often more sensitive.
Methods: In order to estimate the influence of age on the development of early cellular epigenetic alterations involved in carcinogenesis, peripheral blood mononuclear cells sampled from 90 patients from three age classes (25–30, 50–55 and 75–80 years old) were ex vivo exposed to urban $PM_{2.5}$.
Results: Particles exposure led to variations in telomerase activity and telomeres length in all age groups without any influence of age. Conversely, $P16^{INK4A}$ gene expression increased significantly with age after exposure to $PM_{2.5}$. Age could enhance *MGMT* gene expression after exposure to particles, by decreasing the level of promoter methylation in the oldest people.
Conclusion: Hence, our results demonstrated several tendencies in cells modification depending on age, even if all epigenetic assays were carried out after a limited exposure time allowing only one or two cell cycles. Since lung cancer symptoms appear only at an advanced stage, our results underline the needs for further investigation on the studied biomarkers for early diagnosis of carcinogenesis to improve survival.

1. Background

Cancer is the leading cause of death between the ages of 60 and 79. More than 50% of all cancers and > 70% of cancer-related deaths occur after 65 years (Howlader et al., 2013). This public health problem is growing due to population aging and increased life expectancy. Lung cancer represents the first cause of death worldwide. Its incidence is steadily increasing among women (3 fold in 20 years), in connection with the rise in smoking, which is the first risk factor. The prognosis remains gloomy with only 14% of 5-years survival, presumably as the first symptoms, thus diagnosis, appear only at an advanced stage of the disease. Therefore, the objective is to propose new markers allowing an earlier diagnosis to improve survival.

Numerous degenerative pathologies such as cancer are influenced or directly caused by genetic and epigenetic modifications that are also found in the biology of aging. The link between aging and cancer is complex, because senescence may, in some cases, protect cells from malignant transformation by triggering apoptosis and, in other cases, increase the carcinogenic risk with the appearance of genetic mutations (Hughes et al., 2002). The cellular senescence is characterized by a permanent cell cycle arrest after a number of divisions, and the appearance of a senescent phenotype involving important cellular

Abbreviations: AGT, O^6-AlkylGuanine DNA alkylTransferase; DLPCB, Dioxin Like PolyChloroBiphenyl; EDTA, EthyleneDiamineTetraAcetic acid; FBS, Fetal Bovine Serum; GC/MS, Gas Chromatography/Mass Spectrometry; HRGC/HRMS, High Resolution Gas Chromatography/High Resolution Mass Spectrometry; IARC, International Agency for Research on Cancer; ICP/MS, Inductive Coupled Plasma/Mass Spectrometry; LDH, lactate deshydrogenase; *MGMT*, MethylGuanine-DNA MethylTransferase; $P16^{INK4A}$, P16 Inhibitor Kinase 4A; PBS, Phosphate Buffer Saline; SEM, Scanning Electron Microscopy; PAHs, polycyclic aromatic hydrocarbons; PM, particulate matter; PCDD/F, PolyChloroDibenzo Dioxins/Furans; PCR, Polymerase Chain Reaction; ROS, Reactive Oxygen Species; RPMI, Roswell Park Memorial Institute medium; RT, Reverse Transcription; SD, Standard Deviation; TSG, tumor suppressor genes

* Corresponding author at: Saint Louis University School of Medicine, Division of Geriatric Medicine, 1402 South Grand Boulevard, Room M238, St Louis, MO 63104, USA.
E-mail address: b.fougere@gmail.com (B. Fougère).

https://doi.org/10.1016/j.exger.2018.05.018
Received 21 February 2018; Received in revised form 11 May 2018; Accepted 25 May 2018
Available online 31 May 2018
0531-5565/

reorganizations and gene expression changes. This replicative senescence comes along with the shortening of telomeres which can be counterbalanced by telomerase activation. Cancer cells exhibit high telomerase activity associated with telomeres presenting frequent cytogenetic abnormalities (Gisselsson et al., 2001). Telomerase activation by the onset of mutations can induce immortal cancerous clone, leading to cellular immortalization. Conversely, a lowered activity leads to a premature telomere shortening, up to the entrance into senescence phase and cell proliferation arrest. The effect of pollutants on the length of telomeres is relatively little documented, and the results of studies are sometimes contradictory.

Some epigenetic modifications described in aging are features of cancer (i.e. decrease in global DNA methylation; increase in promoter-specific CpG islands methylation) (Fraga et al., 2007). Methylation profile of many crucial genes promoters is altered during tumorigenesis, leading on one hand to some oncogenes activation through promoter hypomethylation. On the other hand, promoter hypermethylation leads to extinction of tumor suppressor genes (TSG) (e.g. *P16^{INK4A}*) and DNA repair genes (e.g. *O^6-MethylGuanine-DNA MethylTransferase*, *MGMT*). P16^{INK4A} protein inhibits cyclinD-dependant phosphorylation of Rb, thus preventing G1-S transition. *P16^{INK4A}* is often silenced during carcinogenesis by promoter hypermethylation in CpG islands, resulting in the loss of P16^{INK4A} activity and the consecutive deregulation of cell proliferation. The O^6-AlkylGuanine DNA alkylTransferase (AGT) protein, encoded by *MGMT* gene, is a DNA repair enzyme which fixes O^6-methylguanine, a major mutagenic and cytotoxic DNA lesion produced by various endogenous and exogenous methylating agents (Shiraishi et al., 2000). *MGMT* gene promoter is hypermethylated in approximately 20% of lung cancers (Zöchbauer-Müller et al., 2001). Furthermore, the AGT activity decreases with age (Aoki et al., 1993). Finally, exposure to some environmental toxins (e.g. As, Cr, Ni, radon, smoky coal emissions, urban particulate matter (PM), and metal-rich $PM_{2.5}$) may lead to the inactivation of these genes by promoter hypermethylation (Soberanes et al., 2012). Air pollution and particulate matter itself were recently recognized as carcinogenic to humans by the IARC (group 1) (Loomis et al., 2013). The elderly people experience a complex relationship to the environment since they are more sensitive to changes in the environment, such as climate change with temperature increase, and exposure to toxins and infectious agents. This greater sensitivity could result from a lower physiological reserve capacity and a more slowly responding immune system. Their higher disease burden than people at younger ages makes specific organ systems less able to tolerate stress (Carnes et al., 2014). The elderly adults then suffer adverse health effects, with increased numbers of hospitalizations for cardiorespiratory diseases at lower concentrations of pollutants (Bentayeb et al., 2012). Although health effects of air pollution affect all age groups, there have been recent calls for a focus on air pollution research in the elderly population as they are the principal group at risk and are particularly vulnerable to air pollutants (Argacha et al., 2016; Fougère et al., 2015; Sandström et al., 2003; Wen and Gu, 2012). Among air pollutants, $PM_{2.5}$ refers to particles with an aerodynamic diameter lower than 2.5 μm, thus able to penetrate deeply into the lung and to interact with the alveolo-capillary barrier. $PM_{2.5}$ or its soluble constituents may also join the systemic circulation, and affect other target organs and then lead to pro-inflammatory effects, oxidative stress, and genetic alterations (Nemmar et al., 2002).

The aim of this study was to estimate the influence of age on the appearance of early cellular epigenetic alterations potentially involved in carcinogenesis following cell exposure to urban air pollution PM (Fig. 1). $PM_{2.5}$ was collected in a French seaside city characterized by important industrial activities and heavy motor vehicle traffic. Peripheral blood mononuclear cells (PBMC) were sampled from three age classes before ex vivo exposure to $PM_{2.5}$. Telomerase activity and gene expression modulation of *P16^{INK4A}* and *MGMT* were then analyzed, in order to: (i) determine the impact of air pollution on early events of carcinogenesis; (ii) investigate the influence of age on the cell biological response thanks to the recruitment of people from contrasted ages.

2. Methods

2.1. PM sampling, physical and chemical characteristics

PM was collected in Dunkerque (51°04′N; 2°38′E), an industrialized French seaside City located on the southern coast of the North Sea, using high volume cascade impactor. PM size distribution, evaluated by Scanning Electron Microscopy (SEM), showed size ranging from 0.33 μm to 5 μm with 95% of $PM_{2.5}$. ICP/MS was used to quantify metals. GC/MS allowed to identify PAHs coated onto PM. Dioxins/Furans (PCDD/F) and Polychlorobiphenyls (DLPCB) were analyzed by HRGC/HRMS as previously described (Billet et al., 2008) (Table 1).

2.2. Patients PBMC isolation and exposure to air pollution

2.2.1. Blood sampling

This study was approved by the regional ethical committee (i.e. Comité de protection des personnes, 20th December 2011, ECH 11/03, Lille, France). A total of 90 healthy volunteers (male and female) in three age classes (25–30, 50–55 and 75–80 years old; n = 30/age class) were recruited at the Saint-Philibert and the Saint-Vincent Hospitals (Lille, France). Their informed written consents were obtained prior to blood sampling. The exclusion criteria were the following: smoking or smoking cessation < 10 years, corticosteroids or immunosuppressive treatment, radiotherapy, chemotherapy, workers in metallurgy, petrochemical industry or painters. A complete blood count was performed by Sysmex machine, automatically calibrated at every ignition.

2.2.2. PBMC isolation and culture

Whole blood samples were first diluted 1:1 in PBS (EDTA, 1 mM). PBMCs were then isolated by density gradient centrifugation using Ficoll hypaque solution (Amersham). Isolated cells were cultured under agitation in sterile plastic culture tubes (Dutscher), in RPMI containing: 20% FBS, L-glutamin (2 mM), phytohaemagglutinin (E and L) (5 μg/mL), penicillin (1 IU/mL), and streptomycin (1 μg/mL) (InVitrogen). Cells were maintained at 37 °C, in a humidified atmosphere containing 5% CO_2.

2.2.3. Cytotoxicity dose-response relationship

In order to determine the exposure concentration to use for the study of epigenotoxic effects, two cytotoxicity tests of $PM_{2.5}$ were preliminary performed in 96-well microplate. Isolated PBMCs were exposed at a density of 2.10^4 cells/mL of RPMI to 0, 5, 15, 45 or 135 μg $PM_{2.5}$/mL, during 24, 48, or 72 h. Accordingly, at each time of exposure, PBMCs cultured in 16 wells were unexposed, while PBMCs cultured in 8 wells/concentration were exposed. Unexposed cells were used as negative controls (100% viability) and Triton X-100 2% (v/v)-exposed cells as positive controls (100% mortality). Cytotoxicity was evaluated by studying extracellular Lactate DeHydrogenase (LDH) release in cell-free culture supernatants (Cytotoxicity Detection Kit LDH, Roche Diagnostics) and by studying Mitochondrial DeHydrogenase activity in cells (Cell Proliferation Reagent WST-1, Roche Diagnostics), according to the manufacturer's instructions.

2.2.4. PBMC exposure

About 5 million PBMC suspension were exposed or not to collected $PM_{2.5}$ during 72 h at a concentration of 45 μg $PM_{2.5}$/mL, according to the cytotoxicity results. After 72 h of incubation, cells were centrifuged (500*g*; 10 min; 4 °C). Cell pellets were then washed twice in 5 mL of cold PBS (0.01 M; pH = 7.2), and aliquots were quickly frozen at −80 °C.

2.2.5. Telomerase activity measurement

Telomerase activity was assessed using TRAPEZE RT Telomerase detection kit (Merck-Millipore, France), according to manufacturer's