FEBS Letters 581 (2007) 5315–5320

Ambient particulate matter induces alveolar epithelial cell cycle arrest: Role of G1 cyclins

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Received 14 July 2007; revised 29 September 2007; accepted 15 October 2007

Available online 23 October 2007

Edited by Vladimir Skulachev

Abstract **We hypothesized that the ambient air pollution particles (particulate matter; PM) induce cell cycle arrest in alveolar epithelial cells (AEC). Exposure of PM (25 μg/cm^2) to AEC induced cells cycle arrest in G1 phase, inhibited DNA synthesis, blocked cell proliferation and caused decrease in cyclin E, A, D1 and Cyclin E- cyclin-dependent kinase (CDK)-2 kinase activity after 4 h. PM induced upregulation of CDK inhibitor, p21 protein and p21 activity in AEC. SiRNAp21 blocked PM-induced downregulation of cyclins and AEC G1 arrest. Accordingly, we provide the evidence that PM induces AEC G1 arrest by altered regulation of G1 cyclins and CDKs.**

Keywords: Ambient air pollution particle; Cell cycle arrest CDKs; G1 cyclins; Particulate matter

1. Introduction

Airborne particulate matter (PM 2.5 μm) increases morbidity and mortality from cardiopulmonary diseases resulting in an estimated 500000 deaths each year worldwide [1,2]. PM is genotoxic to alveolar epithelial cell (AEC) by causing DNA damage and apoptosis [3–7]. The biochemical and molecular mechanisms underlying particle-induced cytotoxicity are poorly understood. However, the generation of reactive oxygen species (ROS) is known to mediate PM-induced toxicity to various cell components [3–5]. PM contains transition metals such as Fe, Cu, Ni, V, Co, and Cr, which may induce oxidative damage by generation of ROS [4,6]. While, ROS-mediated activation of transcription factors, such as nuclear factor kappa B (NF-κB) and release of inflammatory mediators such as interleukin (IL)-6, IL-8 and tumor necrosis factor-alpha (TNF-α) may lead to lung injury [7]. Finally, we previously showed that activation of a mitochondria regulated death pathway by augmented oxidative stress caused PM-induced apoptosis in AEC [3–5].

Oxidants generate signals that converge to cause wide range of cellular responses ranging from growth arrest; apoptosis and ultimately necrosis depending on the level of oxidative stress experienced [8–10]. H_2O_2, in particular, is known to induce multiphase cell cycle arrest [9]. However, the cellular responses after PM-induced oxidative stress on cell cycle regulation are not known. Control of cell cycle progression in response to oxidative stress is linked to activation of a checkpoint mechanism operating before entry into the S phase [10]. Progression through the G1 phase and the G1–S transition involves sequential assembly and activation of G1 cyclins and cyclin-dependent kinases (CDKs) [10–12]. After oxidant injury, the rapidity of initiation of type II cell proliferation is crucial for a proper healing, as delay in the reepithelialization process has been implicated in the development of pulmonary fibrosis [3,9]. Therefore, characterization of the mechanisms involved in the block of type II cell replication by oxidants; and the internal and external stimuli that regulate the repair mechanisms appear to be critical for the understanding and management of many lung diseases that are associated with oxidative stress. In this study, we sought to determine whether PM-induces AEC G1 arrest by altered regulation of G1 cyclins and CDKs.

2. Materials and methods

2.1. Particulate matter

The ambient particle (2.5 μm) is a well-characterized Dusseldorf PM provided by the US EPA with known elemental composition comparable to US pollutant [3]. Elemental analyses of the PM were accomplished by infrared or thermal conductivity assays. Particles contain carbon (19.70 ± 2.34%), hydrogen (1.4 ± 0.3%), nitrogen (<0.05%), oxygen (14.12 ± 1.56), sulfur (2.09 ± 0.55%) and ash (63.24 ± 4.19%). Ionizable concentrations of metals include cobalt (103 ± 13 ppm), copper (48 ± 10 ppm), chromium (104 ± 23 ppm), iron (14,521 ± 572 ppm), manganese (21.3 ± 37 ppm), nickel (1519 ± 158 ppm), titanium (131 ± 45 ppm) and vanadium (2767 ± 190 ppm) [3].

2.2. Cell culture

A549 cells were obtained from the American Type Culture Collection and maintained in Dulbecco's modified Eagle's medium (DMEM) containing L-glutamine (0.3 μg/ml), non-essential amino acids, penicillin (100U/ml), streptomycin (200 μg/ml), and 10% fetal bovine serum (FBS; GIBCO) in a humidified 95% air–5% CO_2 incubator at 37 °C. Targeting p21siRNA was done by cell transfection using commercially available p21siRNA duplexes (Santa Cruz Lab) exactly as per the manufactures protocol. After transfection, the cells were synchronized at G0/G1 phase by serum starvation exactly as given below.

2.3. Cell synchronization by serum starvation

Cells were synchronized at G0/G1 phase by serum starvation in DMEM with 0.5% bovine calf serum for 48 h, then 10% serum was added to induce the cells to re-enter the cell cycle [5,9].

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Abbreviations: AEC, alveolar epithelial cells; CDK, cyclin-dependent kinases; PM, particulate matter; ROS, reactive oxygen species

0014-5793/$32.00
doi:10.1016/j.febslet.2007.10.020

2.4. Cell cycle analysis

Cells were synchronized as above, exposed to PM (25 μg/cm^2) with or without 10% FBS, incubated for variable period (0–24 h) and then trypsinized, harvested, washed, resuspended gently in 5 ml of 90% ethanol and fixed at 25 °C for 1 h. Then, cells were incubated with DNase-free RNase A (200 μg/ml) at 37 °C for 1 h, followed by Propidium iodide (10 μg/ml) at 37 °C for 5 min. Cells were separated by sonicating at 20% output level for 15 s using a VirSonic 50 sonicator (Vitis), sorted by fluorescence-activated cell sorter and analyzed using FlowJo (Tree Start). For all of the experiments given below the cells were first synchronized by serum starvation exactly as described above and then treated with PM.

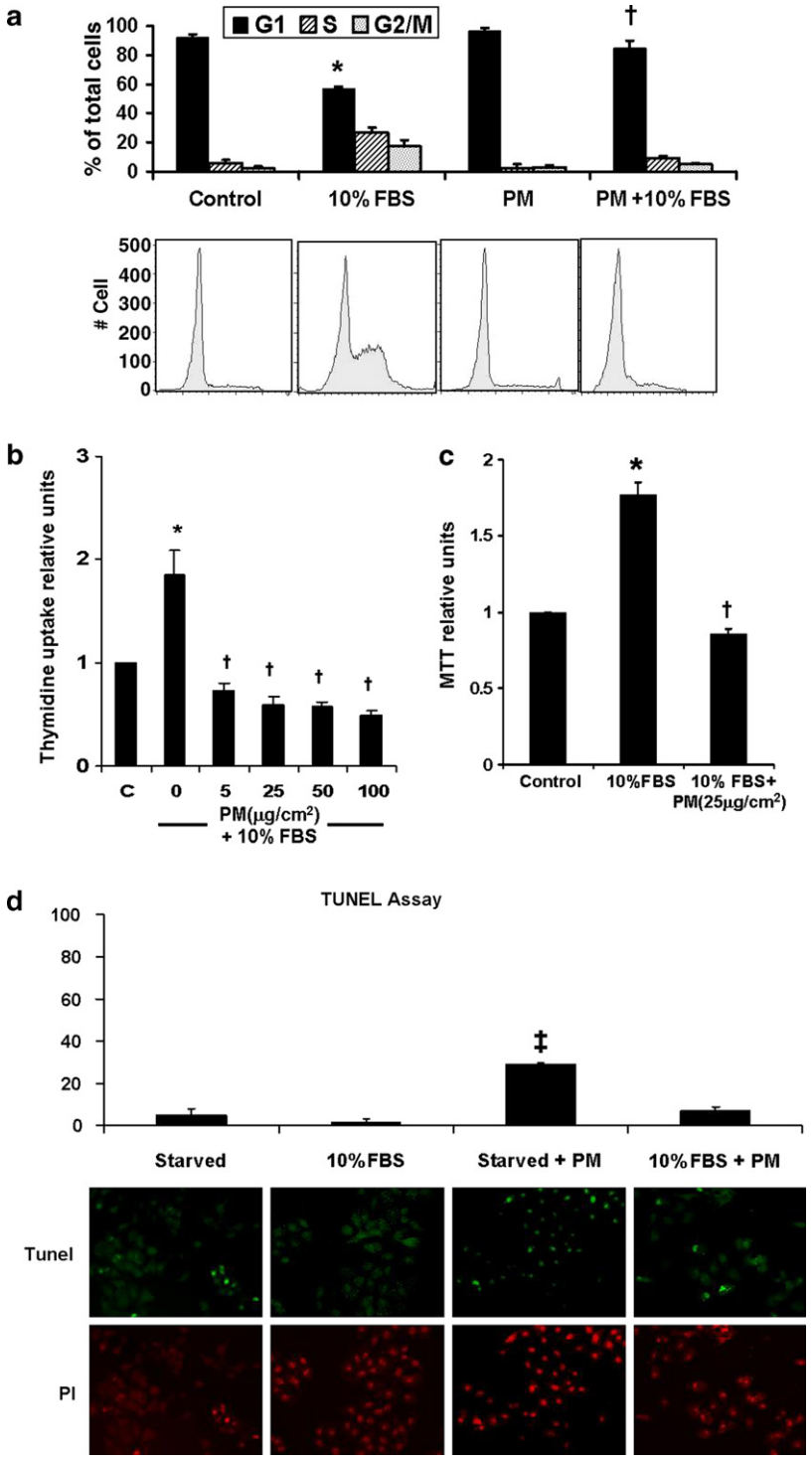

Fig. 1. (a) PM induces alveolar epithelial cell cycle arrest in G1. Cell cycle analysis shows that addition of PM (25 μg/cm^2) induced G1 arrest and prevented serum-induced G1 to S progression in AEC; this effect was seen as early as 4 h. (b and c) PM induced about 2-fold reduction in DNA synthesis and decreases cell survival in cells exposed to PM with 10% FBS as compared to 10% FBS alone. (d) PM in presence of 10% FBS did not induce significant apoptosis as assessed by the TUNEL assay. Means ± S.E.M., $n = 3$. $^*P < 0.05$ control vs. 10% FBS, $^\dagger P < 0.05$ 10% FBS vs. 10% FBS + PM, $^\ddagger P < 0.05$ serum starved vs. serum starved + PM.