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Effects of airborne particulate matter on alternative pre-mRNA splicing in colon cancer cells

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ABSTRACT

Alternative pre-mRNA splicing plays key roles in determining tissue- and species-specific cell differentiation as well as in the onset of hereditary disease and cancer, being controlled by multiple post- and co-transcriptional regulatory mechanisms. We report here that airborne particulate matter, resulting from industrial pollution, inhibits expression and specifically affects alternative splicing at the 5′ untranslated region of the mRNA encoding the bone morphogenetic protein BMP4 in human colon cells in culture. These effects are consistent with a previously reported role for BMP4 in preventing colon cancer development, suggesting that ingestion of particulate matter could contribute to the onset of colon cell proliferation. We also show that the underlying mechanism might involve changes in transcriptional elongation. This is the first study to demonstrate that particulate matter causes non-pleiotropic changes in alternative splicing.

1. Introduction

Airborne particulate matter (PM) has been linked to many adverse health effects, in particular on cardiorespiratory diseases (Araujo and Nel, 2009; Lipsett et al., 2011), type II diabetes (Meo et al., 2015) and cancer (Krewski et al., 2009; Raaschou-Nielsen et al., 2011; Loomis et al., 2013). Jeddah, the second largest city in Saudi Arabia, is a setting of high PM exposure. Components of the PM from Jeddah were characterized by Khoder et al. (2012). PM collected from Jeddah was found to increase expression of genes whose products belong to pathways associated with disease including metabolic syndrome and atherosclerosis both in vitro (Sun et al., 2012) and in vivo (Brocato et al. 2014). Recent studies revealed that the average concentrations of PM10 in Jeddah were clearly much higher than the limit established by the World Health Organization (WHO) of 50 μg/m3 for 24 hours (Alghamdi et al. 2015a, Khoder et al. 2012). The elemental composition of PM10 revealed that tey are rich in Cr, Mn, Sr, Co, As, Pb, Cd, Ni and V (Khoder et al., 2012, Alghamdi et al., 2015a). There are also data for one group of specific organic compounds, the polycyclic aromatic hydrocarbons in Jeddah (Alghamdi et al., 2015b). These do not comprise a large proportion of particulate matter mass, but are important due to the carcinogenicity of some members of this group of compounds.

Colorectal cancer (CRC) is a serious life-threatening health problem in Saudi Arabia. It is the number one cancer in the male Saudi population. CRC has been related to various dietary, life style and environmental factors. Among these, high consumption of red meat, low intake of folate, smoking, lack of activity, and exposure to environmental pollutants were found to play a significant role in colorectal carcinogenesis. Particulate air pollution is derived primarily from vehicular traffic and burning of fossil fuels, and it may also arise from the desert environment. Accordingly, elevated risks of colon cancer were found among petrol station/automobile repair workers, workers exposed to asbestos, soot, cutting fluids/oils and combustion gases from coal/coke/wood (De Verdier et al., 1992). Recently an increase in mortality due to CRC in towns lying in the vicinity of metal production and processing installations

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was reported (García-Pérez et al., 2010).

There is a misconception that the main regulatory event determining cell differentiation and functioning is the differential control of gene expression at the level of transcription. The role of alternative pre-mRNA splicing in the definition of cell behavior in response to environmental cues is greatly neglected. We know now that the regulation of gene expression not only implies the knowledge of which genes are turned on and which ones are turned off, but also implies the detailed knowledge of which alternative splicing isoforms are produced from those genes that are "on" (for a review see Kornblihtt et al., 2013). Therefore, the purpose of the present study is to determine whether airborne particulate matter has an effect on gene expression in colon cancer cells at the level of alternative splicing and whether this effect is of a general nature or specific to certain genes. We found that exposure of colon cancer cells in culture to PM10 specifically affects alternative splicing of the pre-mRNA for the bone morphogenetic protein BMP4. It does this by favoring a splicing isoform that does not alter the encoded amino acid sequence but that reduces the overall expression of the protein. This is consistent with previously reported evidence (Lombardo et al., 2011) that inhibition of BMP4 expression may contribute to the onset of colon cancer.

2. Materials and methods

2.1. Particle sample collection

Dust samples were collected from the campus of King Abdulaziz University, located in south Jeddah. Particles were collected for 48 h on 5300 Polypropylene filters using a Staplex high volume air sampler (Staplex Air Sampler Division, USA) with PM10 inlet (serial no. 2840) at a fixed flow rate of 900 l/min.

Particle extraction: particles of 10 μm diameter or less (PM10) were extracted from polypropylene filters using a modified aqueous extraction protocol (Duvall et al. 2008). Briefly, each filter was wetted with 25 ml of 70% ethanol followed by sonication in 100 ml of distilled water for 2 h. The particles were dried by lyophilization, then weighed and stored at −80 °C.

2.2. Cell culture and transfection

HEK293T (human embryonic kidney) and HCT116 (human colon cancer) cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g of glucose and 10% fetal bovine serum (Gibco, Life Technologies, Gaithesburg, MD, USA) at 37 °C.

Cell viability was determined by trypan blue exclusion; cells were plated at a density of 5.10^4/well in 24-well plates and cultured for 48 h with the stated amount of PM10 particles. After incubation, the cells were released with trypsin/EDTA, incubated with trypan blue, and counted using a hemocytometer.

Camptothecin (Sigma-Aldrich, St. Louis, MO, USA) 6 μM was added 4 h before cell harvest. For siRNA experiments, cells were transfected with siLuc (5′CUUACGCUGAGUACUUCGA3′), at a final concentration of 20 nM. Approximately 48 h after transfection, RNA was extracted for RT-PCR studies.

2.3. RNA extraction and RT-PCR

Total RNA was extracted with Tri-Reagent (Ambion) according to the manufacturer's instructions. This method combines phenol and guanidine thiocyanate in a monophasic solution to rapidly inhibit RNase activity.

One microgram of RNA was reverse transcribed with the M-MLV reverse transcriptase (Invitrogen) and oligo-dT primer, and the cDNA was amplified with human BMP4 primers 5′CGAGAAGGCAGAGGAGGAG3′ and 5′CAAACTTGCTGGAAAGGCTC3′. Radioactive PCR conditions and alternative splicing evaluation through native polyacrylamide gel electrophoresis were done as previously reported (de la Mata et al., 2003). To measure BMP4 mRNA levels through real time PCR, cDNA was amplified using primers 5′CACTGGTCTTGAGTATCC3′ and 5′CTGCTGAGGTTAAAGAGG3′ mapping to BMP4 exons 3 and 4 respectively. Amplification of the control mRNA of Hsp90 was carried out using primers 5′CCAAAAAGCACCTGGAGATCA3′ and 5′TGTCGGCCTCAGCCTTCT3′.

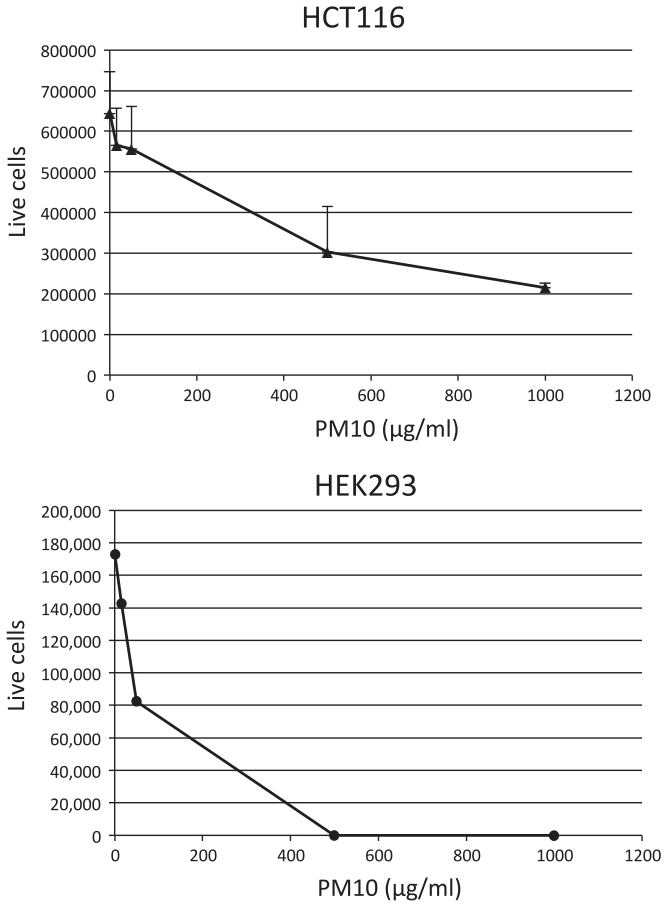

Fig. 1. Effect of PM10 particles on cell viability of the human colon carcinoma cell line HCT116 and the human embryo kidney transformed cell line HEK293. Cells were incubated with increasing amounts of PM10 for 48 h and counted as described in Section 2. HCT116 data correspond to average and SD of three independent experiments. HEK293 data correspond to a single representative experiment.

2.4. Pol II elongation measurement

An adaptation of the method developed by Singh and Padgett (2009) was used. A DRB (5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole) treatment of 300 mM for 5 h was necessary in order to fully block endogenous transcription of the human fibronectin gene (FN1). For the CPT-treated cells, CPT 1 mM was added 30 min before the DRB wash and remained until cell harvest. Total RNA was extracted as mentioned above. Reverse transcriptase reaction was initiated with random decamers. Quantification of the pre-mRNAs was performed on the fibronectin gene by real-time PCR with an amplicon spanning the exon 1/intron1 junction. The primers used were FNe1F: 5′TGGCTGTCAGTCAAAGCAAG3′ and FNi1R: 5′CAGCTGGTTTCTCTCAGTAAAGC3′. Results were expressed in relation to the pre-mRNA value of cells never treated with DRB.