International Journal of Hygiene and Environmental Health 215 (2012) 522–535

Contents lists available at SciVerse ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.de/ijheh

Effects of alpha particle radiation on gene expression in human pulmonary epithelial cells

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ARTICLE INFO

Article history:
Received 10 May 2011
Received in revised form 27 March 2012
Accepted 15 April 2012

Keywords:
Microarray
Epithelial cells
Alpha particles
Radon
Gene expression

ABSTRACT

The general public receives approximately half of its exposure to natural radiation through alpha (α)-particles from radon (^{222}Rn) gas and its decay progeny. Epidemiological studies have found a positive correlation between exposure to ^{222}Rn and lung carcinogenesis. An understanding of the transcriptional responses involved in these effects remains limited. In this study, genomic technology was employed to mine for subtle changes in gene expression that may be representative of an altered physiological state. Human lung epithelial cells were exposed to 0, 0.03, 0.3 and 0.9 Gy of α-particle radiation. Microarray analysis was employed to determine transcript expression levels 4 h and 24 h after exposure. A total of 590 genes were shown to be differentially expressed in the α-particle radiated samples (false discovery rate (FDR) ≤ 0.05). Sub-set of these transcripts were time-responsive, dose-responsive and both time- and dose-responsive. Pathway analysis showed functions related to cell cycle arrest, and DNA replication, recombination and repair (FDR ≤ 0.05). The canonical pathways associated with these genes were in relation to pyrimidine metabolism, G2/M damage checkpoint regulation and p53 signaling (FDR ≤ 0.05). Overall, this gene expression profile suggests that α-particle radiation inhibits DNA synthesis and subsequent mitosis, and causes cell cycle arrest.

Introduction

It has been established that radon (^{222}Rn) gas and its progeny constitutes about half of the natural radiation dosage to which the average person is exposed (Caswell and Coyne, 1990). One of the most abundant sources of ^{222}Rn gas is from the decay series of uranium (^{238}U), which is found naturally and ubiquitously in varying concentrations in bedrock throughout the earth. Epidemiological studies conducted among uranium mine workers, as well as animal cancer studies, have found a strong correlation between ^{222}Rn exposure and an increase in the development of lung cancer (Kennedy et al., 2002; Samet et al., 1991; Stather, 2004; Darby et al., 2005). These studies have triggered considerable concern about similar effects on the general population (Neuberger and Gesell, 2002) and have prompted investigations into the possible effects of residential ^{222}Rn exposure and associated lung cancer risk over the past decade. The results from these studies have yielded mixed data, with some studies suggesting a positive association between ^{222}Rn concentrations and lung cancer, while others show equivocal or negative results (Samet and Eradze, 2000). More recently, combined analysis of data from residential ^{222}Rn case–control studies have shown that there is a measurable risk of lung cancer at ^{222}Rn levels as low as 100 Bq/m^3 (Darby and Hill, 2003; Krewski et al., 2005). The suspected association between high concentrations of ^{222}Rn and the incidence of lung cancer has drawn concern about elevated ^{222}Rn concentrations indoors, which in conjunction with other carcinogens and aerosols like tobacco smoke may have compounding health effects.

Radon gas can decay into solid radioactive particles, which emit α-particle radiation (Appleton, 2007; Iwaoka et al., 2007; Al-Zoughool and Krewski, 2009). Most ^{222}Rn gas inhaled is immediately exhaled, however, if decay occurs in the lungs, the resulting solid radioactive particles can settle onto bronchial epithelial cells. This can lead to irradiation of the lungs with α-particles, which can

Abbreviations: ^{241}Am, americium; ^{137}Cs, cesium; ^{222}Rn, radon; ^{214}Po, polonium; ^{238}Pu, plutonium; FC, fold change; MD, Mylar based plastic dishes; FBS, fetal bovine serum; TBS, triphosphate buffered saline; TST, TBS serum triton; PBS, phosphate buffered saline; EDTA, ethylenediaminetetraacetic acid; Cy, cyanine; qPCR, quantitative real time polymerase chain reaction; CT, comparative threshold; DCT, delta CT; FDR, false discovery rate; NTP, nucleotide triphosphate; OD, optical density; AROS, array ready oligio sets; IPA, ingenuity pathway analysis; PA, pathway analysis.

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1438-4639/$ – see front matter.
http://dx.doi.org/10.1016/j.ijheh.2012.04.004

cause DNA damage. The majority of the energy deposited in biological systems from the α-particles can produce a high density of ionizations and deliver large localized energy of about 10–50 cGy (National Research Council, 1999). An earlier study by Henshaw et al. (1990) revealed there is a correlation between national concentrations of ^{222}Rn and the incidence of childhood cancers. Many studies have been conducted which look at the cytogenetic effects of α-particle radiation (Jostes, 1996). A number of these *in vitro* and *in vivo* studies have shown that α-particle exposure can lead to mutagenic changes including large deletions, frameshift and base-change mutations (Lutze et al., 1992; Taya et al., 1994; Brooks et al., 1993; Schwartz et al., 1990; Shadley et al., 1991).

Only a few studies have been conducted on the transcriptional changes induced by α-particle exposure. These studies mostly looked at the transcriptional levels of specific genes, including those associated with apoptosis, DNA damage repair and oncogenesis. In a recent study by Li et al. (2007) BALB/c mice were exposed to ^{222}Rn inhalation of 100 Bq/m^3, 12 h/day for up to 62.5 days. The authors reported increased transcript levels of E-cadherin and down-regulation of transcription of replication protein A1 and casein kinase 1 delta, in bone marrow cells. Human breast cancer cells (MCF-7) exposed to ^{222}Rn gas for three days, at doses between 0.6 and 8.3 mGy, resulted in under-expression of bax and bc1-2 genes, over-expression of bcl-x_L, and significant levels of alternative mRNA splicing of the same gene bcl-x_S (Soto et al., 2006). In another study, conducted by Turtoi et al., lymphocytes isolated from peripheral blood of two healthy human donors were exposed for 30 min to ^{211}At at doses ranging from 0.05 to 1.6 Gy (Turtoi and Schneeweiss, 2009). Following an incubation period of 2 h, the expression of 18 genes was tested for differential expression. Of these, seventeen genes were shown to be up-regulated. There is, however, limited data on the effects of α-particle exposure on genome wide transcriptional expression changes.

The aim of the present study was to identify genes responding to low to moderate doses of α-particle radiation in a dose- and time-dependent manner as they would represent reliable α-particle radiation responsive genes. For this purpose, human-derived lung epithelial cells were exposed to α-particles emitted from Americium (^{241}Am) electroplated discs, at doses 0.03 Gy, 0.3 Gy and 0.9 Gy, harvested 4 and 24 h post-exposure and analyzed using microarray technology for differential changes in gene expression patterns relative to a control group. Lung epithelial cells were chosen as the epithelial lining of the airways forms the first line of defense against toxic agents in the air and a number of studies have highlighted the importance of this cell-type in responding directly to air borne particulate matter (Fujii et al., 2001). Several of these studies have shown a strong response in epithelial cells leading to changes in cellular morphology (Bayram et al., 1998), release of inflammatory cytokines (Ohtoshi et al., 1998), and alterations in cellular functions (Stringer and Kobzik, 1998), following air-borne particle exposure. Therefore, epithelial cells serve as an appropriate model cell type for studying the biological effects α-particle exposure. Genomic technology was used, as the expression of thousands of genes in a genome can be quantified in a single experiment. This technology provides a means for assessing changes in gene expression patterns in cells and thereby providing insights into the oncogenic pathways induced by carcinogens, such as ^{222}Rn (Ying and Sarwal, 2009).

Materials and methods

Cell exposure and harvesting

Human derived lung epithelial cells (A549) obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA), were maintained in a humidified incubator at 37 °C, 5% CO_2, 95% air in 75 cm^2 flasks (T-75). The cells were grown to confluence for 2–3 days in F-12K medium, containing 10% fetal bovine serum (ATCC) and then seeded into 35 mm culture dishes containing 2 layers of 2.5 μm-thick Mylar films. The seeding density was at 8×10^5 cells/dish with 2 mL of culture media containing 100 units/mL of penicillin and 100 μg/mL streptomycin. Cells were cultured to about 90% confluency, then exposed to α-particle radiation at doses of 0 (control), 0.03, 0.3 and 0.9 Gy using ^{241}Am electroplated discs having an activity level of 66.0 kBq ± 3% (dose rate of 0.98 ± 0.01 Gy/h, LET of 127.4 ± 0.4 keV/μm). The absorbed dose of α-radiation to which cells were exposed was calculated using the GEANT4 v.9.1 Monte Carlo toolkit (Beaton et al., 2011). For the α-particle exposures, cells were cultured in thin Mylar based plastic dishes (MD) (Chemplex Industries, Palm City, FL, USA), which allowed penetration of the α-particles. The cells were harvested 4 or 24 h after exposure, allowing for transcriptional changes to occur. For each exposure group, a total of 5 independent experiments were conducted. The cells were washed with phosphate buffered saline (PBS) and harvested using 200 μL 0.25% trypsin–EDTA (Invitrogen Corporation, Carlsbad, CA, USA). Media was added to bring the volume to 700 μL with 30 μL being used to determine the cell viability and concentration (Strauss, 1991). All cells remained 98% viable. The remaining cells were centrifuged at 1000 rpm for 5 min, decanted, and resuspended in 350 μL of lysis buffer, provided by Qiagen's RNeasy Mini kit. The cells were stored at −80 °C until RNA isolation (Qiagen Inc., Mississauga, ON, Canada).

RNA isolation and cRNA generation

The frozen lysates were pipetted onto a QIAshredder spin column, and the total RNA was extracted using the RNeasy Mini kit according to the manufacturer's instructions (Qiagen Inc.). Additionally, Qiagen's On-Column RNase-free DNase was used to eliminate possible DNA contamination. The concentration and quality of the RNA sample isolation was determined through spectrophotometric means (optical density (OD) ratio of A260:A280), and using the Agilent 2100 Bioanalyzer, following the manufacturer's instructions (Agilent Technologies, Mississauga, ON).

Agilent's Low RNA Input Fluorescent Linear Amplification Kit was used to generate fluorescently labeled cRNA, from the total RNA, following the manufacturer's protocol (Agilent Technologies, Mississauga, ON). The mRNA from the total RNA was primed with the (d)T-T7 primer and amplified using MMLV-reverse transcriptase into (5′–3′) cDNA. Cyanine-3 labeled (3′–5′) cRNA was generated from the cDNA, using T7 RNA polymerase and isolated using the Qiagen's RNeasy Mini kit following the manufacturer's protocol (Qiagen Inc.).

Hybridization

The concentration of Cy3-labeled cRNA, and the amount of incorporated Cy3 dye was quantified using the NanoDrop1000 spectrophotometer following manufacturer's protocol (NanoDrop Technologies Inc. Wilmington, DE, USA). Cy3-labeled cRNA, with a total incorporation of ~40 pmol of Cy3, was fragmented and hybridization buffer was added following the Agilent hybridization protocol (Agilent Technologies). The hybridization solution was pipetted onto the OpArrays Homo sapiens (human) array ready oligo sets (AROS) V4.0 microarray slides, which contain 35,035 oligonucleotide probes, representing approximately 25,100 unique genes and 39,600 transcripts excluding control oligos (Operon Biotechnologies Inc., Huntsville, AL, USA). The microarrays were hybridized for 17 h in a 60 °C rotating chamber, followed by washes using non-stringent buffers, dried using pressurized air, and scanned using Scanarray GX (Perkin Elmer; Woodridge ON), with