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Gene expression responses in human lung fibroblasts exposed to alpha particle radiation

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ABSTRACT

This study examined alpha (α-) particle radiation effects on global changes in gene expression for the purposes of identifying potential signaling pathways that may be involved in Radon (^{222}Rn) gas exposure and lung carcinogenesis. Human lung fibroblast cells were exposed to α-particle radiation at a dose range of 0–1.5 Gy. Twenty-four hours post-exposure, transcript modulations were monitored using microarray technology. A total of 208 genes were shown to be dose-responsive (FDR adjusted $p < 0.05$, Fold change > |2|) of which 32% were upregulated and 68% downregulated. Fourteen of the high expressing genes (>|4| fold) were further validated using alternate technology and among these genes, *GDF15* and *FGF2* were assessed at the protein level. *GDF15*, a known marker of lung injury, had expression levels 3-fold higher in exposed cell culture media, 24 h post-irradiation as detected by ELISA. Further, pathway analysis of the dose-responsive transcripts showed them to be involved in biological processes related to cell cycle control/mitosis, chromosome instability and cell differentiation. This panel of genes with particular focus on *GDF15* may merit further analysis to determine their specific role in mechanisms leading to α-particle induced lung carcinogenesis.

1. Introduction

In recent years, α-particle radiation has become an increasing public health concern. Despite their limited penetrating power, α-particles have a strong capacity to produce an intensely damaging biological response due to their dense ionization tracks (Goodhead, 2010). For this reason, α-particle ingestion (e.g. Polonium-210) or inhalation (e.g. radon gas and its daughter progeny, isotopes present in cigarette smoke) may have detrimental effects leading to potential long-term health consequences (Al-Zoughool and Krewski, 2009). Of particular concern are urban areas where high indoor radon levels can generate absorbed doses that are well above the average of 2.4 mSv/year (Hendry et al., 2009). Further to being an environmental concern, α-particle emitters like Americium-241, Plutonium-238 and Polonium-210 have been identified by the American Nuclear Regulatory Commission and the Secretary of Energy (www.energy.gov/media/RDDRPTF14MAYa.pdf) as some of the most probable isotopes to be used in radiological dispersal devices (e.g. dirty bombs). The action of α-particle radiation on cells is also important from the perspective of radiation protection. As new technological developments emerge in the area of radiation therapy involving the use of α-particles, there is growing concern regarding exposure of cancer patients to this high LET radiation during therapy (Wang et al., 2010; Difilippo et al., 2003 and Kry et al., 2005).

Although numerous epidemiological studies have shown considerable evidence associating exposure to α-particle radiation with adverse health effects (e.g. lung cancer) (Kennedy et al., 2002; Samet et al., 1991; Stather, 2004; Darby et al., 2005 and Neuberger and Gesell, 2002), clear evidence-based studies to support these claims have yet to be fully elucidated. Currently there is a vast amount of data showing α-particle radiation effects on cytogenetic markers including γ-H2AX formation, chromatid exchange, and chromosomal aberrations (Hu et al., 2013; Loucas et al., 2013 and Jostes, 1996), however there are limited studies that have

Abbreviations: (^{241}Am), americium; (^{222}Rn), radon; (^{226}Ra), radium; (^{210}Po), polonium; (MD), mylar based plastic dishes; (FBS), fetal bovine serum; (PBS), phosphate buffered saline; (α), alpha; (MD), mylar dish; (RPMI), Roswell Park Memorial Institute; (ANOVA), analysis of variance; (qRT-PCR), quantitative real-time polymerase chain reaction; (Ct), comparative threshold; (FC), fold change; (RDDs), radiological dispersal devices; (LET), linear energy transfer; (IPA), ingenuity pathway analysis; (FDR), false discovery rate; (ELISA), enzyme-linked immunosorbent assay; (RIN), RNA Integrity Number.

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examined global changes at the transcriptional level. Certain cellular responses to direct ionizing radiation exposure are often mediated through modulation of gene expression. Furthermore, these studies are central to delineating signaling pathways and showing the effects mechanism for radiation-induced adverse responses. To date, numerous studies in the field of low-linear energy transfer (LET) ionizing radiation have effectively made use of microarray technology to understand bystander effects (Kalanxhi and Dahle, 2011; Kalanxhi and Dahle, 2012 and Chaudhry and Omaruddin, 2011) and radio-sensitivity and resistance (Reviewed in Oh et al., 2012). In the present study, the effects of α-particle radiation were examined at the transcriptional level in a relevant normal human cell line. Previous work from Health Canada's laboratory has shown some promising findings in transformed cell-lines (Chauhan et al., 2012a, 2012b) where protein secretion, DNA damage response and gene expression modulations were examined after exposure to α-particles in monocytic and epithelial cells. At higher doses of α-particle radiation ($\geqslant$0.5 Gy), significant modulation in protein and gene expression leading to DNA damage and cellular apoptosis was observed. To further complement this work and address knowledge gaps concerning the response of primary human cells, the current study examined the transcriptional and secretory protein modulations following α-particle radiation exposure (*in vitro*) in normal human lung fibroblasts. Therefore, the outcome of this work will enhance our understanding and knowledge with regards tothe mechanistic effects of α-particle exposure on human health.

2. Materials and methods

2.1. Cell culture and irradiation

Human primary lung fibroblasts (HFL-1) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). HFL-1 cells were maintained in a humidified incubator (37 °C, 5% CO_2/95% air) in 75 cm^2 tissue culture flasks (Costar, Cambridge, MA, USA). The cells were grown to confluence for 2–3 days and cultivated in F-12K medium (Invitrogen Canada, Burlington, ON Canada) containing 10% Fetal Bovine Serum (FBS) (Sigma–Aldrich Canada, Oakville, ON, Canada). A total of 1.0×10^6 cells were seeded into 5 mL of culture media containing 100 units/mL of penicillin and 100 μg/mL of streptomycin (Invitrogen Canada Inc.). The cells were exposed to α-particle radiation at doses ranging from 0.0 (control) to 1.5 Gy, using ^{241}Americium (^{241}Am) electroplated discs (Eckert and Ziegler Isotope Products Ltd., Valencia, CA, USA) having an activity level of 66.0 kBq ± 3%. The exposure time was calculated based on a dose rate of 0.98 ± 0.01 Gy/h and an energy deposition of 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). X-ray irradiations were performed using the X-RAD 320 X-ray irradiation system (Precision X-ray, Inc., North Branford, CT, USA) at a matched dose rate of 0.98 ± 0.05 Gy/h. Following exposure, cells were allowed to recover for 24 h before being harvested. A total of 6 independent experiments were conducted. Cell viability data was measured using the Fluorescein Diacetate Assay (Strauss, 1991).

2.2. RNA extraction

Twenty-four hours following exposure to α-particle radiation or negative control conditions the cells were washed with phosphate buffered saline (PBS) and harvested using 200 μL 0.25% trypsin-EDTA (Invitrogen Corporation, Carlsbad, CA, USA). Medium was added to bring the volume to 700 μL. 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's RNeasy Mini kit; Qiagen Inc, Mississauga, ON). The frozen lysates were thawed and 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).

2.3. cDNA generation

All extracted RNA samples were determined to be of good quality (RNA Integrity Number (RIN) = 10) with minimal degradation and stored at −80 °C until further analysis. Samples with a RIN value of greater than or equal to 8.0 were deemed to be acceptable for analysis. An input of 200 ng of total RNA was used for whole genome analysis following the Illumina(r) Whole Genome Expression Profiling Assay Guide (11317302 Rev. A). Samples were hybridized on Illumina human-12 v2 RNA BeadChips. BeadChips were imaged and quantified with the Illumina iScan scanner and data was processed with Illumina GenomeStudio v2010.2.

2.4. Statistical analysis

Data pre-processing was carried out within GenomeStudio, where the intensities were averaged per probe/gene. Normalization of dataset was conducted in GeneSpring (version GX 11.5). Intensities were normalized to the 25th percentile. Intensities were log2 transformed and a two tailed T-test were performed. The variance was not assumed to be the same between the groups. Multiple testing using Benjamini & Hochberg false discovery rate (FDR) correction was applied to the *p*-values in order to obtain robust responding gene targets.

2.5. Quantitative real time-polymerase chain reaction (qRT-PCR)

Selected genes identified by microarray analysis as displaying statistical significance, with fold changes of 2 or higher and for which validated primers were available were further assessed by qRT-PCR. Total RNA (100 ng) isolated from cells were reverse transcribed into complementary DNA using the RT^2 First Strand Kit (SABiosciences Corp., Frederick, Maryland, USA). Gene profiling was performed according to the manufacturer instructions using custom RT^2-profiler PCR arrays (SABioSciences Corp.). Reactions were prepared in 96-well plates and were performed in a spectrofluorometric thermal cycler (Biorad iCycler; Hercules, CA). The relative expression of each gene was determined by using the comparative threshold (Ct) method (Livak and Schmittgen, 2001). Analysis of qRT-PCR expression profiles and statistical analysis of data was assessed using the super array biosciences web portal for data analysis of their products. (SABiosciences http://www.sabiosciences.com/pcr/arrayanalysis.php).

2.6. Protein validations

Twenty-four hours following exposures, supernatants from exposed (0.5, 1.0, 1.5 Gy) and control samples were analyzed for secretion levels of GDF15 and FGF2 using commercially available ELISA set (Invitrogen). ELISA was performed according to the manufacturer's instructions. All samples and standards were performed in duplicate. Statistical analysis was performed using a one-way ANOVA with Dunnett's post hoc test