INTRODUCTION

In this work, we evaluated the differential effects of low- and high-LET radiation on 3-D organotypic cultures in order to investigate radiation quality impacts on gene expression and cellular responses. Current risk models for assessment of space radiation-induced cancer have large uncertainties because the models for adverse health effects following radiation exposure are founded on epidemiological analyses of human populations exposed to low-LET radiation. Reducing these uncertainties requires new knowledge on the fundamental differences in biological responses (the so-called radiation quality effects) triggered by heavy ion particle radiation versus low-LET radiation associated with Earth-based exposures. In order to better quantify these radiation quality effects in biological systems, we are utilizing novel 3-D organotypic human tissue models for space radiation research. These models hold promise for risk assessment as they provide a format for study of human cells within a realistic tissue framework, thereby bridging the gap between 2-D monolayer culture and animal models for risk extrapolation to humans. To identify biological pathway signatures unique to heavy ion particle exposure, functional gene set enrichment analysis (GSEA) was used with whole transcriptome profiling. GSEA has been used extensively as a method to garner biological information in a variety of model systems but has not been commonly used to analyze radiation effects [1]. It is a powerful approach for assessing the functional significance of radiation quality-dependent changes from these datasets where the changes are subtle but broad, and where single gene-based analysis using rankings of fold-change may not reveal important biological information.

METHODS

2-D Cell Culture

Normal immortalized human esophageal epithelial cells (EPC2-hTERT) were cultured in Keratinocyte Serum Free Media and FEP3 cells (normal human esophageal fibroblasts) were cultured in DMEM (10% FBS+ pen/strep).

3-D Organotypic Culture

Organotypic model of human esophageal epithelium. Cultures were grown in DMEM followed by Epidermalization media (DMEM, Ham’s F12 and supplements). (A) Schematic diagram of an organotypic culture showing individual layers. (B) Stroma-equivalent collagen/fibroblast base prior to epithelial seeding., shown in 24 mm transwell inserted into a 6-well plate; (C) H&E stained section of an organotypic culture (image from www.histology.net); (D) H&E stained section of a formatin-free paraffin-embedded slice from a 15-day organotypic culture of EPC2-hTERT cells grown in our laboratory at JSC; (E) A section from a culture immunostained for the basal marker KRT14 (red) and the suprabasal marker KRT13 (green); nuclei are counterstained with DAPI (blue).

Irradiation

Low-LET irradiation studies were conducted with a 1000-curie gamma source. High-LET particle was conducted at the NASA Space Radiation Laboratory at Brookhaven National Laboratory during campaigns NSRL-10A.

Gene Arrays and Analysis

Illumina expression microarrays, (R2: 44 K; R4: 64 K; R13: 14 K, R12: 12 K; R11: 11 K), arrays that provide coverage for 47,000 transcripts and splice variants were used to profile global gene expression changes in EPC2-hTERT epithelial cells grown in 3-D organotypic culture at 72 hrs post-exposure to 137Cs gamma-rays (100 rad) or 3-D Organotypic Culture 48Ti 350 MeV/u (30 rad) particle radiation. These exposures represent isotoxic brachytherapy and space radiation, respectively.

RT-qPCR

Analysis of genes was performed using oligonucleotides primers designed with Primer3. Primer design for each gene was performed using the software. Primers were ordered from SA Biosciences. Cells were cultured in KSFM and EPI or from 3-D epithelial layers. Fold changes in relative gene expression were calculated using the fold-change method.

RESULTS

Clonogenic Survival

EPC2-hTERT cells were irradiated at 100 rad and 300 rad and plated in 6-well plates with 100–200 cells per well. A total of 10 wells were plated at each dose level. Cells were irradiated with 137Cs gamma-rays (200 rad) or 48Ti 350 MeV/u (100 rad) particle radiation. Cells were cultured in 2-D monolayer culture and animal models for risk extrapolation to humans. To identify biological pathway signatures unique to heavy ion particle exposure, functional gene set enrichment analysis (GSEA) was used with whole transcriptome profiling. GSEA has been used extensively as a method to garner biological information in a variety of model systems but has not been commonly used to analyze radiation effects [1]. It is a powerful approach for assessing the functional significance of radiation quality-dependent changes from these datasets where the changes are subtle but broad, and where single gene-based analysis using rankings of fold-change may not reveal important biological information.

Top-Ranking Data Sets from GSEA Analysis

We identified 45 statistically significant gene sets at 0.05 p-value cutoff, including 14 gene sets common to gamma and titanium irradiation, 19 gene sets specific to gamma irradiation, and 12 titanium-specific gene sets.

CONCLUSIONS

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REFERENCES