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 studying 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 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 FEF3 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) Stromal-equivalent collagen/fibroblast base prior to epithelial seeding, shown in 2 cm2 transwell inserted into a 6-well plate; (C) H&E stained section of an organotypus (image from www.histology.net); (D) H&E stained section of a formalin-fixed paraffin-embedded slice from a 15-day organotypic culture of EPC2-hTERT cells grown in our laboratory at JSC; (E) A section from a similar 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 100 keV/cm gamma source. High-LET particle was conducted at the NASA Space Radiation Laboratory at Brookhaven National Laboratory during campaigns NSRL ’12A.

Gene Arrays and Analysis

In vitro specimens (HT2 immortalized Seadship; Illumina, Inc.) 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 350 MeV/u (30 rad) particle radiation. These exposures represent isotoxic doses based on clonogenic survival assays performed on cells cultured in 2-D monolayers. Raw Illumina data were preprocessed using R statistical package dChip. RT-qPCR data normalized to GAPDH. Primers were ordered from SA Biosciences.

Clonogenic Survival

EPC2 cells were seeded at 500 cells per 60 mm dish and frozen. Upon thawing, cells were plated at increasing densities (500 plates per density). At 12 days, cells were fixed and stained with 0.1% crystal violet in 10% formalin and colonies containing more than 50 cells were counted.

RESULTS

2-3 Cell Death

Equitoxic cell death at 30r and 100r for Ti and Cs

Up and Down Regulated Gene Sets

Top-Ranking Data Sets from GSEA Analysis

γ-Titanium Common

Dose (rad)

Clonogenic Survival

Top-Ranking Data Sets from GSEA Analysis

Gene Interaction Networks

GSEA Gamma Genes

Enrichment Maps

Gene Specific

REFERENCES


CONCLUSIONS

We identified 45 statistically significant gene sets at 0.05 q-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. Common gene sets largely align with DNA damage, cell cycle, early immune response, and inflammatory cytokine pathway activation. The top gene set enriched for the gamma-irradiated samples involved KSHV pathway activation, while the top ranking gene set identified for the titanium exposure contains genes whose expression is increased in TNF-activated cells (Phong, TIF Targets Up). TNF is a multifunctional pro-inflammmatory cytokine that controls diverse biological processes, dictating cell killing by activation of apoptotic programs but also acting as a strong survival signal through NFκB, p38, and JNK-dependent pathways.

Another difference noted for the high-LET samples was an apparent enrichment in gene sets involved in cycle cycle/mitotic control. It is plausible that the enrichment in these particular pathways results from the complex DNA damage response following high-LET irradiation resulting from lack of repair where repair processes are not completed during the same time scale as the less complex damage resulting from low-LET radiation.

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REFERENCES


ANOTHER DIFFERENCE NOTED FOR THE HIGH-LET SAMPLES WAS AN APPARENT ENRICHMENT IN GENE SETS INVOLVED IN CYCLE CYCLE/MITOTIC CONTROL. IT IS PLAUSIBLE THAT THE ENRICHMENT IN THESE PARTICULAR PATHWAYS RESULTS FROM THE COMPLEX DNA DAMAGE RESPONSE FOLLOWING HIGH-LET IRRADIATION RESULTING FROM LACK OF REPAIR WHERE REPAIR PROCESSES ARE NOT COMPLETED DURING THE SAME TIME SCALE AS THE LESS COMPLEX DAMAGE RESULTING FROM LOW-LET IRRADIATION.