**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 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 Epithelialization media (DMEM, Haim’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 3-D form transwell inserted into a 6-well plate. (C) H&E stained section of human esophagus (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 137Cs cesium 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**

To probe 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 48T,350 MeV/u (30 rad) particle radiation, these exposures represent isotropic doses based on clonogenic survival analyses performed on cells cultured in 2-D monolayers. Raw Illumina data were preprocessed using R statistical package for background correction and normalization. GSEA (Gene Set Enrichment Analysis) was done using a t-test for ranking differentially expressed genes for background correction and normalization. GSEA (Gene Set Enrichment Analysis) was done using whole transcriptome profiling.

**RESULTS**


**Clonogenic Survival**

**Gamma/Titanium Common Genes**

**Gamma/Titanium Common Genes**

**Top-Ranking Data Sets from GSEA Analysis**

**Up and Down Regulated Gene Sets**

**Gene Interaction Networks**

**Enrichment Maps**

**CONCLUSIONS**

- We identified 45 statistically significant gene sets at a 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 KRAS pathway activation, while the top ranking gene set identified for the titanium exposure contains genes whose expression is increased in TRF-targeted cells (Phong_TNF_TARGETS_UP).
- Another difference noted for the high-LET samples was an apparent enrichment in gene sets involved in cytokine-mediated cytoprotection. It is plausible that the enrichment in these particular pathways results from the total DNA damage resulting from high-LET exposure 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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