RADIATION QUALITY EFFECTS ON TRANSCRIPTOME PROFILES IN 3-D CULTURES AFTER CHARGED PARTICLE IRRADIATION

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INTRODUCTION

Current risk models for assessment of space radiation-induced cancer have large uncertainties because they 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 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. In this work, we evaluate 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. Functional enrichment analysis was used with whole transcriptome profiling to identify biological pathway signatures unique to heavy ion particle exposure. 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

Illumina platform (HT12 Expression Beadchip; 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 ^{137}Cs gamma-rays (100 rad) or ^{48}Ti 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 for background correction and normalization. Gene set enrichment analysis (GSEA) was done using a *t-test* for ranking differentially expressed genes under gamma and titanium irradiation [1] and run for three functional annotation datasets independently. These gene sets were obtained from the Molecular Signature Database (MsigDB), including curated gene sets, gene ontology gene sets, and oncogenic signature gene sets. The statistical significance of up- or down-regulated gene sets were assessed by permuting gene set 1,000 times as recommended in the GSEA reference manual. Gene sets were then ranked by two different approaches (FDR q-value based and leading-gene based ranking) and an average ranking was calculated for each gene set which represent the extent of dysregulation of gene sets by the two radiation types.

DISCUSSION

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 KRAS pathway activation, while the top ranking gene set identified for the titanium exposure contains genes whose expression is increased in TNF-treated cells (Phong_TNF_Targets_Up) [2]. TNF is a multifunctional, proinflammatory 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 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.

REFERENCES

[1] Subramanian et al (2005) PNAS 103, 15545–50.
[2] Phong et al (2010) *Mol Cell Biol* 30, 3816.