HZE Particle Radiation-Induced Gene Expression Changes Specific to Particle Type in Human Bronchial Epithelial Cells and Promoted Anchorage Independent Growth in Synergy with Oncogenic KRAS and p53 Suppression

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Using non-oncogenically immortalized human bronchial epithelial cells (HBEC3KT) and a syngeneic cell line that exhibits p53 suppression and active oncogenic KRAS expression (HBEC3KT_KRASp53), we examined the early transcriptional response to HZE particle irradiation and the ability of HZE particles to induce anchorage independent growth. During 4 NSRL runs, we have collected samples that have been irradiated with Fe, Si and \(\gamma\)-ray. Illumina whole genome microarrays were used to profile gene expression changes at 1, 4, 12 and 24 hours after radiation. At least 3 independent experiments were performed at different NSRL runs for each radiation quality. Our results for HBEC3KT showed distinct expression profiles in response to different radiation qualities. This was evident by: 1) unsupervised cluster analysis where samples were grouped by radiation quality; 2) the sources of variation from ANOVA modeling identified radiation quality as the major source of gene expression differences; 3) The number of differentially expressed genes unique to each radiation quality were more numerous than those commonly changed genes across all radiation qualities; 4) a gene signature based upon the response to radiation of different quality yielded a very high accuracy when used to predict radiation quality in independent HBEC samples. Interestingly, for HBEC3KT, despite the differential response at the gene level, gene ontology analysis revealed that the majority of altered functional and signaling pathways after exposure to radiation of different qualities were highly common.

The syngeneically modified HBEC3KT_KRASp53 cells exhibited a significantly higher baseline for cellular transformation as determined by growth in soft agar when compared to the parental HBEC3KT cell line. The number of colonies formed in soft agar by HBEC3KT_KRASp53 cells was 4 times more than HBEC3KT cells. Gene expression analysis comparing these two cell lines identified HIF-1\(\alpha\), which is known to promote anchorage independent growth \cite{1}, as activated in the HBEC3KT_KRASp53 cells. We have also examined the effect of radiation on anchorage independent growth of these two cell lines. The cells were plated into soft agar between 2-3 weeks after radiation. HBEC3KT cells did not show significant increase in soft agar clones compared to un-irradiated controls. The number of soft agar clones for HBEC3KT_KRASp53 doubled in \(\gamma\)-ray irradiated samples (1 Gy) and more than 4 times in HZE irradiated samples (1 Gy) when compared to un-irradiated HBEC3KT_KRASp53 cells. Our data indicated that ionizing radiation further enhanced the soft agar growth in the modified cells but not in the normal parent cells with the HZE radiation being more efficient at cellular transformation than \(\gamma\)-rays. Gene ontology analysis identified several pathways, all involved in anchorage independent growth, that were significantly activated in cell cultures that showed an increased ability to generate soft agar colonies. These pathways included the mTOR, IGF-1, RhoA and ERK/MAPK signaling pathways \cite{2-5}. These results suggest that ionizing radiation given as a single acute dose was not sufficient to induce anchorage independent growth in normal HBEC3KT cells. However, a single dose of radiation was sufficient to enhance anchorage independent growth in HBEC3KT_KRASp53 cells. Associated with this increase in transformation is the enhancement of several molecular pathways which were non-responding in irradiated HBEC3KT cells but robustly activated in irradiated HBEC3KT_KRASp53 cells. This suggests that ionizing radiation, especially HZE particle irradiation, promoted the carcinogenic process in synergy with oncogenic KRAS and p53 suppression in lung epithelial cells.

References: