Cell and Animal Models of Lung Cancer: Response to Radiation

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Lung cancer is the leading cause of cancer death in both women and men in the USA and in the Western world. While lung cancer is caused by smoking exposure in 85% of people, 15% of lung cancer cases arise in life time never smokers (~25,000 patients per year). Thus lung cancer in never smokers is the 7th leading cause of cancer related deaths in the USA. Likewise, 50% of all new lung cancer cases occur in former smokers (quitting over 5 years before). In all of these scenarios a major underlying question has been the role of other environmental carcinogens especially environmental radiation. One source of this is radon gas in the environment including radon gas exposure in homes with alpha particles (which represent high LET radiation). Thus, the interaction of radiation in the environment on Earth including from alpha particles may be important in the development of lung cancer and quantitation of the effect of such radiation on lung epithelial cells is of major health importance. Radiation is a known carcinogenic influence but the molecular events in lung epithelial cells in response to radiation need to be determined. A key element of this is the impact of radiation on lung epithelial cells with preneoplastic lesions.

Human Lung Epithelial Cells to Study Radiation Effects

We have developed a human bronchial epithelial cell (HBEC) culture system to study the effects of genetic and environmental changes on human lung epithelium. These HBECs are immortal, clonable, can be genetically manipulated, do not form colonies in soft agar and are not tumorigenic. We used the human bronchial epithelial cell line HBEC3 together with isogenic derivatives (p53 knockdown and KRAS^{V12} mutant over-expression) and treated them with various doses of ^{56}Fe, ^{28}Si and gamma irradiation. The cells were collected at various time points following irradiation (0, 1, 4, 12, 24 hr, and 1, 2, 3, and 4 months), the RNA was extracted and gene expression was measured (Illumina Whole Genome microarrays: 47,000 probes). Acute changes ($\leq$24 hr time points) were found in gene expression following exposure of the HBECs to each radiation type. These gene expression signatures were distinct for gamma irradiation compared to HZE particle irradiation (^{56}Fe, ^{28}Si) as well as for the various HBEC isogenic genetic variants. This panel of cells exposed to different type and doses of irradiation showed distinct clustering patterns on unsupervised hierarchical clustering, principal component and regression analysis. This was true for equivalent dose and equivalent survival exposures. Four major canonical pathways were found to be differentially regulated when 1 Gy of ^{56}Fe was

compared to 3 Gy of low LET radiation. Bayesian network analysis of HBEC3 and the RAS/p53 variant also revealed unique gene nodes which, when combined with other analysis, suggests genetic background influences on gene expression after HZE particle exposure.

We also determined the rate of cellular transformation as a function of radiation dose and quality, using growth of cells in soft agar as an initial end point. The HBECs were irradiated with either low LET radiation or ^{56}Fe or ^{28}Si particles. Cell cultures were maintained for at least 4 months post-IR and at monthly intervals the cells were examined for the ability to grow in soft agar. The ability of low LET radiation to induce transformation beyond the basal level was very limited. However, when HBEC 3KT cells were irradiated with ^{56}Fe or ^{28}Si, there was a significant increase in transformation which quickly declined as a function of dose, likely due to cell death mitigating any transformative events. The biological and gene expression results are being integrated into a model to provide quantitative risk assessment for developing lung cancer after radiation exposure.

Mouse Models of Lung Cancer to Study Radiation Effects

Expression of oncogenic K-ras in the mouse lung initiates the formation of lesions that mimic early lung cancer progression in humans and thus permits evaluation of lung cancer progression in an *in vivo* model. We hypothesized that if space radiation exposure influenced lung cancer progression, then utilization of a lung cancer susceptible mouse model would enhance the detection of even subtle alterations. We sought to determine if ^{56}Fe particle radiation or x-ray exposure affected initiation or progression of lung cancer in the lung cancer susceptible *K-ras*$^{LA1/+}$ mouse that without radiation survives less than two years. Each radiation type was administered whole body either as a single 0.1Gy - 1.0 Gy dose, or in fractionated dose of 0.2 Gy per day for 5 days or 0.1 Gy per day over a 12 day period. We determined that irradiation did not impact the number or size of lung tumors in these animals. However, survival differences were apparent and dependent on radiation type. Animals irradiated with ^{56}Fe particles (both single and fractionated doses) had a significantly decreased maximum lifespan compared to unirradiated controls. In contrast, x-ray irradiation did not affect maximum lifespan. Histopathological analysis of tumors from *K-ras*$^{LA1/+}$ mice irradiated with a fractionated dose of ^{56}Fe particles demonstrated a dramatic progression in grade of lesion compared to all other groups. In all established mouse models of lung cancer, including the *K-ras*$^{LA1/+}$ mouse, advanced lesions such as adenomas with regions of atypia and well-differentiated adenocarcinomas are rare suggesting that intact barriers to invasive cancer remain. Importantly, *K-ras*$^{LA1/+}$ mice that received a fractionated 1.0 Gy dose of ^{56}Fe particles (but not single dose) showed a significant increase in the number of invasive adenocarcinomas. The malignant adenocarcinomas observed resembled the clinical features of malignant lung cancer in humans. Such mouse models of cancer susceptibility may help reduce the uncertainties in the projection of cancer or other health risks for sustained human exposure to space radiation.