

THE RADIORESPONSE OF NORMAL HUMAN BRONCHIAL EPITHELIAL CELLS TO CHARGED PARTICLE EXPOSURES OF INCREASING MASS, ENERGY AND LET.M. D. Story^{1,2}, L-H. Ding¹, S. Park¹, and J. D. Minna^{2,3,4}

¹ Department of Radiation Oncology, ² Simmons Comprehensive Cancer Center, ³ Hamon Center for Therapeutic Oncology Research and ⁴ Department of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd. Dallas, TX USA 75390

Cellular transformation and oncogenesis: We have used a model of non-oncogenically immortalized normal human bronchial epithelial cells to determine the response of such cells to particles found outside the protection of the earth's electromagnetic field. We have identified an enhanced frequency of cellular transformation for both ⁵⁶Fe and ²⁸Si (1 GeV/n) that is maximal (4-6 fold) at 0.25 Gy and 0.40 Gy, respectively. Anchorage-independent growth, as determined by growth in soft agar, was used as a measure of cellular transformation. At 4 months post-irradiation 38 individual soft agar clones were isolated. These clones were characterized extensively for cellular and molecular changes. Gene expression analysis suggested that these clones had down-regulated several genes associated with anti-oxidant pathways including GLS2, GPX1 and 4, SOD2, PIG3, and NQO1 amongst others. Examining clones for ROS-mediated DNA damage by gH2Ax and 53BP1 foci identified higher levels of gH2Ax with stable levels of 53BP1, suggesting higher levels of ROS-mediated DNA damage. DNA repair pathways associated with ATM/ATR signaling were also upregulated. However, these transformants do not develop into tumors when injected into immune-compromised mice, suggesting that they have not progressed sufficiently to become oncogenic. Therefore we chose 6 soft agar clones for continuous culture for an additional 14 months. Amongst the 6 clones, only one clone showed any significant change in phenotype. Clone 3kt-ff.2a, propagated for 18 months, were 2-fold more radioresistant, had a shortened doubling time and the background rate of transformation more than doubled. Furthermore, the morphology of transformed clones changed from very small foci to clones of 1-2 mm in diameter. Clones from this culture are being compared to the original clone as well as the parental HBEC3KT and will be injected into immune-compromised mice for oncogenic potential.

Transcriptional response to HZE radiations: We have performed gene expression profiling in HBEC3KT to discern the transcriptional responses to HZE particles at different energies and LETs including ¹²C (250 MeV/n, 14 keV/μm), ¹⁶O (120 MeV/n, 43 keV/μm), ¹⁶O (1000 MeV/n, 14 keV/μm), ²⁸Si (150 MeV/n, 110 keV/μm), ²⁸Si (1000 MeV/n, 43 keV/μm), ⁴⁸Ti (380 MeV/n, 150 keV/μm), ⁴⁸Ti (1000 MeV/n, 110 keV/μm), ⁵⁶Fe (150 MeV/n, 360 keV/μm), ⁵⁶Fe (300 MeV/n, 240 keV/μm), ⁵⁶Fe (600 MeV/n, 170 keV/μm) and ⁵⁶Fe (1000 MeV/n, 150 keV/μm). Based upon particle characteristics these particles were binned into 7 different LET values. Amongst them, 4 values (14 keV/μm, 43 keV/μm, 110 keV/μm and 150 keV/μm) were produced from multiple particles of different energies. Protons at 220 MeV/n and 1000 MeV/n and γ-ray were also used. The cells were irradiated at 1Gy for all the radiation types. Cells were harvested at 24 hours after radiation together with non-irradiated control samples. RNA was extracted and gene expression profiling was performed using the Illumina HT-12 v4 expression BeadChip. The data was summarized using GenomeStudio and MBOB for background subtraction and normalization. Batch effects were corrected based on the NSRL run date for each irradiation. Our results showed a correlation of transcriptome profiles with LET values. This is supported by unsupervised clustering and Source of Variation analysis. A 4-way ANOVA model using variables of ion specie, energy, LET and NSRL run as a random factor generated a list of 636 genes that significantly affected by the factor of LET (p < 0.01). Further study is ongoing to analyze the molecular functions of these genes.

Summary: At the transcriptional level, HBEC cells respond roughly as a function of the LET of the incident particle. Furthermore, HZE particles initiate the carcinogenic process at very low doses. These transformed HBECs exist in an environment of oxidative stress, however, they are not oncogenic.