# THE CONTRIBUTION OF NON-TARGETED EFFECTS IN HZE CANCER RISK

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## BACKGROUND

The NYU/LBNL/UCSF NSCOR provides experimental and modeling studies to define the efficiency and physiological context in which high LET radiation increases epithelial cancer risk. We hypothesize that both targeted and non-targeted effects contribute to radiation carcinogenesis. Targeted effects of ionizing radiation result from interaction of energy with DNA, leading to DNA damage responses and changes in genomic sequence. On the other hand, poorly understood NTE alter phenotype and multicellular interactions that could contribute to cancer. We hypothesize that the greater carcinogenic risk from high LET irradiation is due to non-targeted effects promotion of targeted radiation effects. Our studies use several models in which *Trp53* is modified. *Trp53* is a tumor suppressor that is regularly mutated in cancers, and is often referred to as the guardian of the genome due to the multiplicity of anti-proliferative effects that maintain genomic integrity under genotoxic stress conditions. These data provide a comprehensive analysis of LET dependence for solid tumor frequency in different tissues, which are integrated using computational modeling and systems biology to identify key events.

## RESULTS

Project 1 uses a radiation-genetic chimera in which unirradiated *Trp53* null mammary epithelium transplanted to wildtype hosts that were previously irradiated. Palpable cancers arising in HZE irradiated mice grew faster than those in γ-irradiated mice, both of which grew faster than those in sham-irradiated mice. Tumor estrogen receptor (ER) status is prognostic in breast cancer; 37% (19/51) of tumors in sham-irradiated mice were ER-negative, which increased significantly (Chi Square, $p<0.001$) in both low LET irradiated (74%, 17/23) and high LET irradiated (58%, 37/64) hosts. Cytokeratins (CK) mark mammary epithelial cells; myoepithelial cells are CK14 positive while luminal cells are CK18 positive. Most cancers arising in sham-irradiated mice were positive for both keratins while significantly more ($p<0.05$) tumors arising in irradiated mice were negative for keratin 14. Tumors that arose in irradiated mice were nearly twice (44% vs 28%) as likely to metastasize to the lungs and were also more likely to recur after resection. Thus tumors affected only by radiation NTE, i.e. arising in irradiated mice, were far more aggressive than those from non-irradiated mice.

Project 2 uses Trp53 heterozygote mice and *Trp53* ΔP mice that bear a germline deletion of a small N-terminal proline-rich region of *Trp53*. *Trp53* ΔP mice do not develop spontaneous tumors; yet a wide range of solid tissue tumors, including those of the liver, lung and kidney, develops upon exposure to either low or high LET radiation. Homozygous *Trp53* ΔP, wildtype and *Trp53* heterozygote mice on a 99.9% pure FVB/N background were exposed to 50cGy of either 600 MeV/amu $Fe^{56}$ particles or γ-radiation. Tumor latency in *Trp53* ΔP and *Trp53* heterozygote mice was significantly reduced following exposure to high LET radiation compared to the same genotype exposed to low LET (coxPH test $p=0.0068$ and $p=0.0004$ respectively). Of note, there is a negligible impact on tumor-free survival when either *Trp53* ΔP or *Trp53* heterozygote mice are exposed to the same radiation quality (coxPH test low LET irradiation $p=0.668$, high LET irradiated $p=0.448$). In contrast to prior experience with low LET radiation, a high number of mammary tumors (49%) and skin carcinomas (11%) develop in *Trp53* heterozygote mice following exposure to high LET radiation. This suggests a radiation-quality specific shift in tumor spectrum. Further investigation of the potential mechanism by which exposure to high LET radiation causes this shift in spectrum and reduced latency is currently underway.

*In silico* modeling of mammary morphogenesis in Project 3 uses agent-based modeling (ABM) to examine the relative contribution of NTE and TE to mammary tumor incidence as a function of radiation quality. The contribution of TE, i.e. radiation-induced mutations, was simulated in the mammary ABM by adding the possibility of epithelial agents producing mutated progeny and modeled using Monte Carlo schemes of DNA damage as function of radiation quality. Deletion probabilities for three classes were tuned so that frequency of *in silico* tumor development matched empirical incidence and latency of tumors in the radiation chimera mammary model. Simulations show that increased stem cell frequency in irradiated mammary gland accelerated tumor formation *in silico*, as observed *in vivo*. Improved understanding of radiation carcinogenesis achieved by defining the relative contribution of non-targeted effects carcinogenic effects and identifying the genetic determinants of the high LET cancer susceptibility will help reduce uncertainties in radiation risk assessment.