Alterations in mitochondrial gene copy numbers following irradiation in radiosensitive mice.

Sumita Raha, Qiong Wang, Emily Mirkin, M. Beau Wanzer, Tatjana Paunesku and Gayle Woloschak

Department of Radiation Oncology, Feinberg School of Medicine, Northwestern University, 303 E Chicago Ave, IL 60611

We have developed a quantitative real-time PCR approach to evaluate ratios of gene copy numbers in mitochondrial and nuclear DNA. We hypothesized that the gene copy number ratios (GCNR) will be modulated in response DNA damage caused by irradiation as well as other types of stress. As a proof of principle, we did a short term experiment with acute 5Gy gamma-ray irradiation of radiosensitive wasted mice and their normal littermates. Animals were sacrificed 24h post exposure and the tissues harvested included several whole organs. Primary organs affected by wasted gene mutation are central nervous system and muscle, therefore, brain tissue served as a type of "primary stress" control, while kidney, spleen and thymus show signs of stress associated with wasted disease caused by loss of innervation.

The gene copy number ratios in all tissues were between 200 and 500, as evaluated by the following four pairs of mitochondria or nucleus encoded genes for mitochondrial proteins: ND1 and NDUFV1, COX1 and COX6b, Mt CyB and CyB5b and Mt atp6 and atp5a1. These GCNRs correspond to 400-1000 mitochondrial genomes per cell depending on the tissue/cell type and irradiation treatment. The GCNRs in different tissues of healthy and wasted, non-irradiated and irradiated mice showed genotype, organ and treatment dependent differences

Analysis of the GCNR revealed that wasted mice have different baseline GCNRs compared to their healthy littermates. The differences were marked not only in brain (tissue affected in wasted genotype disease) but also in kidney, spleen and thymus—the tissues affected solely by loss of innervation. Moreover, short term response to radiation treatment in radiosensitive wasted mice was often unique as well. The GCNR of the pair of COX genes (COX1 and COX6b) in wasted mice irradiated with 5Gy showed a decrease in brain while in healthy irradiated mice the GCNR increased in every tissue tested post irradiation. For the pair of ND genes (ND1 and NDUFV1), the significant GCNR increase was found in thymus of both wasted and healthy mice; moreover the GCNR of ND genes increased in response to radiation in spleen of healthy mice and brain of wasted mice. The GCNR of the pair of cytochrome b genes (Mt CyB and CyB5b) showed significant increase in all tissues from irradiated wasted mice as well as thymus and kidney of healthy irradiated animals, while their brains showed a GCNR decrease. Finally, for the ATP gene pair (Mt atp6 and atp5a1) the GCNR in wasted mice irradiated with 5Gy showed a decrease in brain and increase in kidney, while the GCNR in healthy irradiated mice showed increases in all tissues except brain. When the GCNR were evaluated for irradiated mice only, comparisons of wasted to healthy mice showed that there was a higher GCNR for COX gene pair in healthy brains than in brains of wasted mice; a lower ND GCNR in healthy kidney and brain and a lower cytochrome gene pair GCNR in thymus and spleen of healthy mice.

It is possible that the GCNR alterations in wasted mice could be associated with loss of innervation; this finding suggests a role for innervation in regulation of resilience to stress and irradiation. Regardless of the reasons for these differences, however, this pilot experiment showed that the GCNR of gene pairs for mitochondrial proteins is a sensitive and reliable approach to investigate genomic and mitochondrial stress caused by irradiation.

This work was supported by United States Department of Energy grants DE-FG02-04ER63920, 05ER64086, DE-FG02-05ER64100, ER64342, and ER64815.