

**No evidence for *in vivo* induction of genomic instability in bone marrow cells collected from mice exposed to low-dose  $^{137}\text{Cs}$   $\gamma$  rays:** Kanokporn Noy Rithidech<sup>1</sup>, Chatchanok Loetchutinat<sup>1</sup>, Louise Honikel<sup>1</sup>, and Elbert B. Whorton<sup>2</sup>

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Assessment of potential health risks associated with exposure to low-dose radiation (at doses below or equal to 0.1 Gy) is still a challenging public health issue. It is therefore important to improve our understanding of potential induction of genomic instability *in vivo* by this low-dose range because it has been widely suggested that elevation of genomic instability also elevates cancer risk. Although the induction of genomic instability by low-dose radiation has been previously described, reported data were derived from studies conducted under a combination of either *in vivo* irradiation/*in vitro* expression or *in vitro* irradiation/*in vivo* expression systems. For these reasons, to accurately predict risks for late health effects of exposure to low-dose radiation, it is essential to conduct studies using an *in vivo* irradiation/ *in vivo* expression strategy, an approach used in this study.

The overall goal of this project is to determine if low doses of low linear energy transfer (LET) radiation can induce genomic instability *in vivo*. We measured the magnitude of genomic instability (expressed as delayed chromosome instability) in bone marrow cells of two strains of mice with different genetic backgrounds (*i.e.* BALB/cJ and C57BL/6J) following a whole-body exposure to varying doses of  $^{137}\text{Cs}$   $\gamma$  rays (0, 0.05, 0.1, and 1.0 Gy). Bone marrow (BM) cells were collected at different times post-irradiation, *i.e.* 1 hr, 4 hrs, 1 month, and 6 months. The BM cells were selected for the study of the induction of genomic instability by low-dose radiation because they proliferate rapidly and are highly susceptible to the development of radiation-induced acute myeloid leukemia. A total of five mice per dose per strain were sacrificed at each time point for sample collection. The extent and frequency and the type of metaphase chromosome aberrations in bone marrow cells collected from exposed mice at 1 and 4 hrs post-irradiation provide a measure of early response to radiation. The frequency of all types of chromosomal damage at late-times after irradiation offers a measure of *in vivo* induction of genomic instability. In addition, the frequency of stable chromosomal exchanges (*i.e.* translocations) at late time points provides a measure of the potential formation of clones of replicating cells *in vivo* and reflects the fraction of surviving cells that may be at an increased risk for subsequent neoplastic transformation. A three-color fluorescence *in situ* hybridization (FISH) protocol for mouse chromosomes 1, 2, and 3 was used for the analysis of delayed stable chromosomal aberrations in metaphase cells. All other visible chromatid-type aberrations and gross structural abnormalities involving non-painted chromosomes were also evaluated on the same metaphase cells used for scoring the stable chromosomal aberrations of painted chromosomes. Levels of nuclear factor-kappa B (NF- $\kappa$ B, a transcription factor) activation were also determined in cells at 1 and 4 hrs following irradiation (indicative of early responses).

We found that 0.1 and 1.0 Gy (but not 0.05 Gy) of  $^{137}\text{Cs}$   $\gamma$  rays were capable of activating NF- $\kappa$ B in BM cells collected at 1 hr post-irradiation from both strains of mice. We also observed that there was no increase in the frequencies of chromosome aberrations in the group of mice without the signal of early-activated NF- $\kappa$ B (*i.e.* mice exposed to 0.05 Gy of  $^{137}\text{Cs}$   $\gamma$  rays) at any time point included in our study. Instead, a reduction of specific types of chromosomal aberrations was found. The results indicated a link between high levels of NF- $\kappa$ B activation early, after irradiation, and radiosensitivity. Additionally, the finding of NF- $\kappa$ B activation at 1 hr in response to 0.1 and 1.0

Gy (but not 0.05 Gy) of  $^{137}\text{Cs}$   $\gamma$  rays suggests a different *in vivo* molecular mechanism in early response to this very low dose (0.05Gy) of radiation as compared to those in response to 0.1 and 1.0 Gy.

A dose-dependent increase ( $p < 0.001$ ) in the number of cells with chromosomal aberrations was detected as a function of dose at early time post- irradiation in both strains of mice. At late times, there were significant decreases in the chromosome-aberration frequencies in both strains of mice. We also found that the chromosomal aberration frequencies were persistently increased for up to 6 months after exposure of BALB/cJ mice, but not C57BL/6J mice, to 1.0 Gy only. Additionally, there was a trend of persistent elevation in all types of chromosomal damage in cells collected at 6 months post-irradiation from BALB/cJ mice exposed to 0.1 Gy, however this increase was not statistically significant. In summary, our data indicated no evidence for the induction of genomic instability after exposure of BALB/cJ or C57BL/6J mice to low doses (at doses below 0.1 Gy) of  $^{137}\text{Cs}$   $\gamma$  rays.

Overall, the new set of data obtained from our laboratory indicates a link between high levels of NF- $\kappa$ B activation (an early event which follows shortly after irradiation) and radiosensitivity (measured by the magnitude of genomic instability). Our data also show that significant differences in levels of protein expression and biological effects could be elucidated after exposure to high and to very low (0.05 Gy) doses of radiation. These exciting new data suggest that effects of radiation exposure reach detectable significance as the energy of the radiation is increased from 0.05 to 0.1 Gy of  $^{137}\text{Cs}$   $\gamma$  rays. However, only one protein involved in one of the signaling cascades was investigated in our study reported here. To improve our understanding of biological effects of low-dose radiation, it is therefore critically important to further search for protein signatures of *in vivo* response at the whole genome level. *Research funded by DOE Low Dose Grant # DE-FG02-02ER63311.*