Global methylation responses to low dose radiation exposure

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Our goal is to study the mechanisms involved in biological responses to low doses of radiation in vivo in the dose range that is relevant to population and occupational exposures. At high radiation doses, DNA double-strand breaks are considered the critical lesion underlying the initiation of radiation-induced carcinogenesis. However, at the very low radiation doses relevant for the general public, the induction of DNA double-strand breaks will be very rare and it is likely that any carcinogenic response will be dominated more by gene expression changes that can affect cancer progression and promotion. The radiation-induced gene expression changes that have been observed after low dose radiation exposure and the phenomenon of low dose radiation induced genomic instability are likely to be mediated by epigenetic regulation, including both gene-specific changes in DNA methylation at promoter regions as well as genome-wide or 'global' methylation changes.

We have developed a sensitive assay to measure the levels of DNA methylation across the genome by analysing a stretch of DNA sequence within the LINE1 repeat element that comprises a very large proportion of the mouse and human genomes. Using bisulphite modification and quantitative real-time PCR, the method can be used to analyse a very large pool of DNA sequences in parallel from throughout the genome. Using high-resolution melt analysis, the entire pool can be surveyed for sequence changes indicating gain or loss of methylation across more than ten CpG's per amplicon simultaneously. We have validated the ability of the assay to detect global methylation changes induced by the chemical demethylating agent 5'-aza-2`-deoxycytidine where changes from the control as small as 3% can be reproducibly detected. We are now undertaking a range of radiation experiments in mice after high, low and very low doses of X-rays, both after defined periods of time from days-to-weeks after irradiation, as well as longitudinally by serial sampling of peripheral blood (not previously reported for mouse). Two-dose adaptive response experiments are also being performed. We are analysing global DNA methylation changes using the LINE1 high-resolution melt assay in different inbred mouse strains, and in a range of tissues including spleen, liver, kidney, prostate and peripheral blood.

Preliminary results using our assay have shown a difference in the baseline levels of DNA methylation between male and female mice and between different tissues as has been previously documented in the literature. These results further validate the ability of the LINE1 methylation assay to sensitively detect biologically-relevant changes. The initial results of the first radiation experiments suggest no significant short-term changes in global DNA methylation after doses from 10 μGy to 10 mGy, with a transient increase in DNA methylation observed 1 day after a 1 Gy irradiation. If the low radiation doses tested are inducing changes in global DNA methylation, these would appear to be smaller than the natural variation observed between the sexes and following the general stress of the sham-irradiation procedure itself. The results of these experiments will provide mechanistic insight for the gene expressions changes which have been observed following low dose radiation

exposure and provide a sensitive means to detect subtle phenotypic changes at the low radiation doses where levels of direct DNA damage are low.

This research was funded by the Low Dose Radiation Research Program, Biological and Environmental Research (BER), US Department of Energy, Grant DE-FG02-05ER64104 and MN is the recipient of the FMCF/ BHP Billiton Low Dose Radiation Research Scholarship.